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STUDIES ON THE RELATIONSHIP OF SOIL MYCOFLORA TO DISEASE INCIDENCE[1]

Thor Kommedahl and Thomas D. Brock [2]

SUMMARY

Mold counts per gm. of soil were consistently higher in oat than in corn or wheat soils, in assays made in 1952 of 3 field plots, each monocultivated since 1915 to either corn, oats, or winter wheat. Mold counts from wheat were usually lowest of the 3 soils assayed. When monocultivated field soil was transferred to greenhouse flats and reseeded to the 3 crops previously sown in field plots, mold counts again were highest in oat soil.

Wheat sown in the greenhouse on field oat-soil had half as much seedling blight as wheat grown on corn or wheat soil. Corn grew best in oat soil, with about ⅓ as many Fusarium-infected plants, as occurred on corn or wheat soils.

In 2 successive plantings of wheat in greenhouse beds, the percentage of Fusarium-infected plants increased from 30 to 78. Similarly, oats increased from 15 to 48 per cent in numbers of infected plants.

In laboratory and greenhouse tests, the combination of *Gibberella zeae* and *Aspergillus fumigatus* (from oat soil) resulted in fewer blighted corn seedlings than occurred for *G. zeae* alone.

The addition to wheat in greenhouse beds of various soil fungi reduced the number of blighted seedlings from 92 per cent with *Fusarium* spp. alone to 7–51 per cent, depending upon the fungus added.

A number of review articles have been written on the relation of microorganisms to the incidence of root diseases of various crops (9, 14, 16). However, little work has been reported in the United States on the relation of soil fungi and crop sequences to the incidence of seedling blights of cereals caused by species of *Fusarium* or *Gibberella.* Consequently, in this study, experiments were made to find out 1) if continuous cropping to either corn, oats, or winter wheat results in a population of fungi, different in number and kind, for each cereal crop, and 2) what relation this has to seedling blight or Fusarium-infection of corn and winter wheat.

MATERIALS AND METHODS.—All soil samples were taken at a 2-in. depth. Standard soil dilution procedures were followed, the soil being diluted 1:5000 after having been shaken for 15 minutes in sterile water on a mechanical shaker. One ml. of the final dilution was pipetted onto a solid agar surface in a petri plate; the plate was then incubated for 3 days at room temperature. Following incubation, the number of mold colonies that developed was used to estimate the number of molds per gm. of soil by appropriate consideration of the original dry weight of soil and the dilution involved. As a rule, 5 plates were used in replicate for each determination, the average value of these 5 being used to estimate molds per gm. of oven-dried soil. Soil samples stored in a refrigerator (5°C.) for 3 days did not differ significantly, with respect to mold content, from samples kept at room temperature for the same time.

It was essential to know if the techniques used would furnish a reliable mean value of molds per gm. of soil. Theoretically, the distribution of mold colonies on plates should follow a Poisson distribution, since the number of molds in soil is large, and the probability of finding a mold at any one point is small. Consequently a statistical control was run on a large group of typical plate-out data, following the procedures of Eisenhart and Wilson (3), and it was established that the distribution of mold colonies follows that of Poisson. Thus the mean value of replicate plates can be considered reliable (4). To determine whether various mold counts were significantly different, the standard "t" test was used.

The choice of an assay medium is important, for it was necessary to use a medium which would allow suitable growth of fungi but not bacteria. Several different media were tried: soil-extract sucrose-nitrate agar, malt-salt agar, glucose-nitrate agar of Martin (10), but the best was that of Warcup (22), which is a Czapek-Dox agar with the addition of 0.5 per cent yeast extract, acidified to pH 4.0. On this medium bacteria rarely developed; however, at times *Rhizopus* colonies covered the plate and prevented accurate counts of other fungus colonies. By omitting yeast extract, *Rhizopus* failed to develop but bacterial growth was favored, even at this acid pH. Warcup's medium was used, except where noted, for all mold assays reported.

Soils were sampled in 1952, from plots in which oats, corn, and winter wheat were grown each year since 1915. There were small differences in pH among the 3 field plots: 7.25 on oat soil, 6.65 on corn soil, and 7.18 on wheat soil. In all 3 plots, 150 lb. of 0-14-7 were applied per acre per year. No nitrogen had been made available since 1936, when manure was applied. However, volunteer black medick (*Medicago lupulina* L.), which fixes nitrogen, grew in the plots following harvest of oats and wheat.

Soil brought in from the 3 field plots was transferred to deep, bottomless flats (18 in. × 20 in. × 10 in.) buried in greenhouse ground beds. Temperatures for day and night were set at 70° and 60° F., respectively.

The pathogenicity of *Gibberella zeae* (Schw.) Petch when combined with a number of other soil

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[2] Now Microbiologist, The Upjohn Co., Kalamazoo, Mich. The writers appreciate the cooperation of Prof. L. E. Thatcher in work with the Agronomy rotation plots, and the technical assistance of Nancy J. Hockman.

TABLE 1.—*Fungus colonies per gm. of oven-dried soil, isolated on 2 different media, from soil of 3 field plots, each of which had been cropped continuously either to oats, corn, or winter wheat since 1915*

Sampling Date	Medium	*Colonies per gram soil of:* oats	corn	wheat
June 10	Martin[a]	93,100	63,400	23,000
	Warcup[b]	70,500	39,500	38,000
June 19	Warcup	53,000	41,100	29,300
June 24	Warcup	69,000	34,000	38,100
July 3	Warcup	107,200	59,000	.....
Average[c]	Warcup	75,000	46,500	35,100
July 15[d]	Warcup	45,000	29,000	26,000

[a] A glucose-nitrate agar medium plus rose bengal and streptomycin (10).

[b] A yeast-extract-sucrose-nitrate agar medium (Czapek-Dox plus 0.5 per cent yeast extract) (22).

[c] Based upon 26 plates for oat soils, 28 plates for corn soils, and 21 plates for wheat, representing the average of the 4 sampling dates of Warcup's medium. Differences were significant using "t" test.

[d] Data obtained after oats and wheat were harvested; corn was about 3 ft. tall.

fungi was determined with a blotter technique. Seed was placed in the crease of folded blotting paper, the crease being 1 in. from the edge of the paper. Ten corn kernels or 50 wheat grains were placed in each folded blotter. The blotters were then moistened and packed vertically in a tray for 3 days, after which a mycelial-and-spore suspension was poured into the folded blotters. Immediately thereafter, inoculated blotters-with-seedlings were planted in soil of greenhouse ground beds.

The isolate of *G. zeae* used was obtained from a rotted corn stalk and caused stalk rot upon reinoculation in corn. It was found to be pathogenic also to wheat seedlings.

MOLD ASSAYS OF FIELD SOIL.—The number of molds per gm. of oven-dried soil was consistently higher from oat soil than it was from corn or wheat soil, (Table 1). Averaging data from the sampling dates of June 10, 19, 24, and July 3, the highest mold counts per gm. were obtained from oat soil (75,000), less from corn soil (46,500), and least from wheat soil (35,100). These differences were statistically significant by the "t" test.

On June 10, assays were run on 2 media (Table 1); however, mold counts were highest from oat soil on each medium. The differences in mold count from one sampling date to the next, on Warcup's medium, may be accounted for by differences in the environment at the times of sampling.

Influence of field environment.—Mold counts were higher on July 3 (Table 1) than they were prior to that time. These samples were taken following 0.81 in. of rain. However, when the counts were made on July 15, after nearly 1 in. of rain, the mold counts were low. It is possible that rainfall influenced the count of July 3. However, the reduction in count of July 15, following harvest, may have been influenced more by the absence of living plants in the oat and wheat plots. Katznelson (8) and Starkey (17) reported a marked decline in microbial numbers subsequent to the maturity and death of plants.

When assays were made from 2 corn soils, 1 of which was manured annually, 73,000 molds were found per gm. of manured soil, and 29,000 molds per gm. of unmanured soil. Furthermore there was a greater variety of fungi in the manured soil. Manuring may effect growth rate, vigor, and maturity of plants, which in turn may influence mold content of soil.

Undoubtedly, there are many factors that influence mold counts of soils, and no generalizations can be made here as to what these factors are.

Influence of greenhouse environment.—Soil was transferred from field plots to greenhouse flats and seeded to corn, oats, and winter wheat—the same crops to which these soils had been cropped since 1915. On the day of transfer from the field (June 24) mold counts were highest on oat soil, (Table 2). After 2 weeks in the greenhouse the mold count more than doubled in each of the 3 soils. When the plants were 7 weeks old (Aug. 15 in Table 2) assays were again made and the high counts on oat soil recurred. Since in general growing conditions are more favorable in the greenhouse the higher counts may be expected. Despite the different conditions mold counts remained high in oat soil.

TABLE 2.—*Number of fungus colonies isolated on Warcup's medium from soils of 3 field plots, each of which had been cropped continuously since 1915, either to oats, corn, or wheat, then on June 24 transferred to greenhouse flats and there seeded to the same crops*

Date of sampling	Location of soil	*Colonies of fungi per gm. soil from:* oats	corn	wheat
June 24	Field	69,000	34,000	38,000
July 7	Greenhouse	150,000	141,000	103,000
August 15	Greenhouse	90,500	73,500	61,000

RELATIONSHIP OF SOIL HISTORY TO INFECTION.—*Continuous cropping.*—From continuous corn, oats, and winter wheat field plots, soil was transferred to greenhouse flats and seeded to Thorne wheat. About 6 weeks later, there were fewer blighted wheat seedlings in oat soil than occurred in either corn or wheat soils, as determined by necrosis of the crowns and basal stem regions of seedlings. Where 40 per cent of the wheat had seedling blight, incited by *Fusarium* spp., on oat soil, 82 and 84 per cent of the wheat seedlings were subsequently blighted when grown on corn and wheat soils, respectively, based upon about 150 plants per test.

When corn hybrid Ohio C54 was sown in greenhouse flats containing soil taken from field plots, it grew tallest on oat soil and shortest on corn soil (Table 3). Furthermore the roots of corn were most numerous and most vigorous-appearing when corn was grown on oat soil, less so on wheat soil, and least on corn soil. That there could also be an effect on yield is shown by Odland, et al (11), who found that corn yields were

TABLE 3.—*Height of corn in inches and percentages of Fusarium-infected corn plants growing in the greenhouse on 3 soils from field plots previously cropped either to corn, oats or winter wheat continuously since 1915*[a]

Soil previously cropped to	Percentage of Fusarium-infected plants	Plant height in inches[b]
oats	20	51
wheat	65	43
corn	52	36

[a] Based on about 30 plants per test.
[b] Differences were statistically significant.

much higher after an oat crop. As also shown (Table 3), the incidence of Fusarium-infected corn plants was least on oat soil.

Cropping of soil to oats resulted subsequently in more vigorous corn plants with less infection by *Fusarium*. Also, less seedling blight of wheat was obtained on oat soil than on corn or wheat soils.

Two successive crops.—To test whether or not replanting a given cereal would affect the incidence of Fusarium-infected plants in the second planting, wheat was first sown in greenhouse ground beds in soil previously cropped to alfalfa. After about 6 weeks, plants were removed and isolations were made from crown tissues. In the identical furrow from which plants were removed, seeds from the same varieties were replanted and allowed to grow for 6 weeks. Upon isolation from the second crop, *Fusarium* spp. and *Rhizoctonia solani* Kuehn were the fungi most frequently and consistently isolated.

In the first planting 30 per cent of the wheat plants were infected with *Fusarium;* the incidence of infection increased to 78 per cent in the second planting. It was found also that Rhizoctonia infection decreased from 33 to 6 per cent in the second planting. Sanford (15) reports that *R. solani* disappeared in soil when associated with repeated cropping of corn, oats, or wheat.

When oats were replanted in soil previously sown to oats, the percentage of 6-week-old plants infected with *Fusarium* spp. increased from 15 to 48 per cent. These percentages are averages of 3 replicates, 90–115 plants per replicate; the results are statistically significant. It was apparent that the increased incidence of seedling blight resulting from continuous cropping of oats or wheat could occur as early as the second successive crop.

ANTAGONISM OF FUNGI TO G. ZEAE.—*Aspergillus fumigatus* (group spp.) was the most abundant fungus species on oat soil and was rarely isolated from corn or wheat soil. Certain species of *Penicillium* (*P. funiculosum* series) were peculiar to corn soil while *Rhizopus* was found predominantly on wheat soil. None of the frequently-isolated fungi from wheat soil inhibited growth on agar of *G. zeae*, while *A. fumigatus* from oat soil and several *Penicillium* spp. from corn inhibited *G. zeae* considerably. This inhibition of growth or antibiosis was determined visually; no attempt was made to isolate an antibiotic substance.

Corn.—*A. fumigatus* effectively reduced the pathogenic effect of *G. zeae* on corn seedlings. This was determined by transferring disinfected seed of corn to large tubes containing nutrient agar. After several days, *G. zeae* and *A. fumigatus* were introduced into the tubes singly and in combination. Two weeks later, necrosis of the crown was evident on plants in each of 5 tubes where *G. zeae* was present alone, but no necrosis in the 5 tubes with only *A. fumigatus*. With both fungi present, virtually no necrosis was found, indicating that *A. fumigatus* had effectively reduced, if not nullified, the effect of *G. zeae*.

In a second test, autoclaved soil in flasks was inoculated with *G. zeae* plus 1 of each of 5 soil fungi. After 3 days of fungus growth, surface-disinfected kernels were planted in the flasks: 5 seeds per flask, 4 flasks per combination of fungi. With 10-day-old seedlings inoculated with *G. zeae* alone, 89 per cent of the seedlings had blight. However, only 8 per cent of the plants were blighted when *G. zeae* and *A. fumigatus* were together. Four species of *Penicillium*, added singly to *G. zeae*, resulted in the following percentages of seedling blight: 64, 25, 20, and 7 per cent, depending upon the species mixed with *G. zeae*. It was apparent that the virulence of *G. zeae* could be suppressed by the presence of other soil fungi.

In a third test, with steamed soil in greenhouse ground beds the results were less pronounced. The addition of *A. fumigatus* inoculum to that of *G. zeae* resulted in a stand of corn of 90 per cent and a shoot dry-weight of 144 gm. per 100 plants, compared with 82 per cent stand and a shoot dry-weight per 100 plants of 133 gm. for *G. zeae* alone, using the blotter technique of inoculation. Seven other soil fungi added singly to *G. zeae* did not improve stands materially in steamed soil nor result in increased dry weights of plants, although in culture each fungus inhibited growth of *G. zeae*. However, in nonsteamed, alfalfa soil in the greenhouse, it was found that the percentage stand was reduced from 88 in noninoculated soil, to 72 when corn seeds in folded blotters were inoculated with *G. zeae* only. Moreover the stand increased to a high of 98 per cent when *Penicillium* sp. from corn soil was mixed with *G. zeae*. Oven-dry weight of 100 shoots was highest when this same *Penicillium* was mixed with *G. zeae*, 319 gm. compared with 215 gm. for *G. zeae* alone. With 6 other soil fungi combined singly with *G. zeae*, the stand varied from 77 to 92 per cent, and the dry weight per 100 plants was nearly always higher with the combinations than for *G. zeae* alone.

Wheat.—When wheat was sown in soil previously cropped (1 year) to wheat, from which several species of *Fusarium* pathogenic to wheat had been isolated, it was found that 92 per cent of the seedlings were diseased; there was general necrosis of the lower stems plus heavily pruned roots. However, based upon 6 replicates of 50 seeds per replicate and inoculated in

folded blotters with 8 fungi isolated from field plots, the following percentages of diseased seedlings resulted with each fungus added singly to seeds-plus-pathogen: *Aspergillus fumigatus* from oat soil, 51; *Penicillium* sp. from corn soil, 17; *Nigrospora* sp. from wheat soil, 16; *Penicillium lilacinum* Thom from oat soil, 13; *Trichoderma lignorum* (Tode) Harz. from field soil, 10; *Penicillium* sp. from corn soil, 7; and *Aspergillus clavatus* Desm. from unknown field soil, 7. Clearly the combination of certain fungi with a pathogen results in fewer diseased plants than occurs with the pathogen alone.

Discussion.—It was found in this study that there were consistently more molds in soil cropped continuously to oats, than there were from soil cropped continuously to corn or wheat. Furthermore there were fewer Fusarium-infected plants of corn and wheat when the latter were seeded in oat soil than when seeded in wheat soil. It was not established that numbers of molds as such account for the lessened disease. However, these results are in harmony with the work of others in that seedling blight, foot or root rots of wheat or corn are reduced by a crop of oats prior to wheat (1, 12, 14) and prior to corn (11, 13). The increased mycoflora in oat soil over wheat soil found by the writers is in accord with the results of Tyner (18) wherein significantly more fungus colonies were isolated from oat-straw compost than from wheat-straw compost. Waksman and Hutchins (19) found that oat straw rots more quickly than does wheat straw; if so, this suggests an explanation for the higher mold counts on oat soil. Furthermore they reported that wheat straw was higher in lignin, and therefore is more resistant to decomposition by microorganisms.

It is thought by the writers that there is a selective effect of a crop like oats in "building up" a population of molds which may control seedlings or root diseases in the succeeding crop. *Aspergillus fumigatus* from oat soil, for example, may under some conditions be of considerable importance in "protecting" subsequent crops from attack by *Fusarium* or *Gibberella* spp. Others have shown that *Fusarium culmorum* is one of the most abundant organisms to colonize wheat straw (21), and that its virulence on wheat could be suppressed by adding *Trichoderma lignorum* (7). Moreover Henry (6) found that a little soil added to the pathogen, *Fusarium graminearum*, reduced infection and improved stands of wheat. That the effect of crop residues on soil microorganisms last longer than one growing season is not likely, from the work of Dawson, et al (2).

There are certain assumptions underlying any methodology where a quantitative estimate of the population of soil microorganisms is sought. In a given mass of soil it is assumed that there is a definite mass of living material (fungi, bacteria, protozoa, algae, etc.) and that this material is distributed more or less uniformly throughout soil. Granted that it is impossible or highly improbable to sample soil and by some procedure arrive at a figure which definitely states the total amount of living material present, some method must be devised that will give an experimental population approaching a theoretical (or true) population. Obviously it is not possible to know how close to the true population the experimentally determined one is, since the true population is forever theoretical and so, merely an assumption. The alternative is to devise parallel methods and determine which gives the greatest and most reliable value, and then use this method as a standard to ascertain the nature of the soil microorganisms and their fluctutations.

Several workers (5, 20) have outlined various methods used to examine the soil population for fungi and bacteria. Of these the most widely-used and useful is the soil dilution method. Although this is by far the best quantitative method now available, there are several obvious difficulties. At high dilutions the colonies developing on a plate presumably arise from single cells or spores, although hyphal fragments probably give rise also to individual colonies. Thus it is not clear what a given colony on a plate represents in terms of the original soil population. By using a standard dilution and shaking procedure, mycelia would presumably break up to approximately the same degree for each determination. Thus different soil treatments may be compared as to their relative but not absolute mold content.

Since difficulties arise from the choice of an appropriate medium, a reasonable approach is to assay the same soil dilution on a variety of media and select the one which gives the highest counts and the richest variety of organisms. The soil population contains a diverse mixture of types, with varying nutritional requirements, and it is obvious that certain media favor certain types of organisms to the exclusion of others. Therefore no complete solution is possible.

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THE EFFECT OF TEMPERATURE AND RELATIVE HUMIDITY ON THE LONGEVITY OF SPORES OF THE OAK WILT FUNGUS [1]

Edward L. Merek and Charles L. Fergus

SUMMARY

Endoconidia and ascospores lived longest under cool, dry conditions. At 95 per cent relative humidity and 37° C. endoconidia survived less than 3 days and ascospores survived 3 days. At 25 per cent relative humidity both kinds of spores survived for 10 days. At 24° C. the ascospores lived four times as long (232 days) at 10 per cent as they did at 95 per cent relative humidity. At 24° C. and below, endoconidia in a relative humidity of 50 per cent or less were viable for at least 81 days. The endoconidia lived longest (173 days) at 3°–9° C. Ascospores were longer lived than the endoconidia (232 days) under the conditions of the experiment. Wetting the ascospores decreased their longevity (at 24° C.) by ½.

Effective control of oak wilt depends on a clarification of the life history of *Endoconidiophora fagacearum* Bretz. One of the major unsolved problems is the manner of overland long-distance spread. A knowledge of the germination requirements and the longevity of the endoconidia and ascospores should contribute to the solution of this problem.

Insects, birds, and rodents are suspected carriers of the spores. If the spores are carried externally on any of these agents (recent evidence strongly incriminates nitidulid beetles (4, 8) as carriers) the spores must be able to withstand unfavorable environmental conditions and remain viable. The germination requirements have been studied (5) and it appears logical to assume that if viable spores are carried to an infection court, presumably wounds, they will be able to germinate and invade the plant.

The ability of the spores to withstand exposure to a wide range of temperature and humidity was studied, since these 2 factors will largely determine their importance in overland spread of the disease from tree to tree.

MATERIALS AND METHODS.—The endoconidia used were obtained by growing isolate No. P-2 for 3 weeks on Nutramigen[2] agar slants at 24° C. The endoconidia were washed from the surface of the slant with sterile distilled water and adjusted to a concentration of 1 million spores per ml. using a Fuchs-Rosenthal counting chamber. The conidial suspensions were poured into a sterile Petri dish and sterile glass beads were dipped in the suspension, then placed in sterile Petri dishes containing short pieces of bent glass rod to keep the beads separated (Fig. 1, A). The beads were dried for 24 hours at room temperature and then placed in desiccators of various relative humidities.

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[2] Nutramigen is a baby food produced by Mead Johnson and Co., Evansville, Ind. Its use as a nutrient medium was suggested by Dr. G. H. Hepting and Dr. V. M. Cutter, Jr. Sixteen gm. Nutramigen and 17 gm. agar were added to 1 liter of distilled water.

Ascospores were obtained by spermatizing 2-week-old cultures of isolate No. 127, grown on chestnut agar plates, with a conidial suspension of isolate No. 78. The ascospore masses were placed on sterile glass cover slips, which were placed, ascospore mass up, on wire netting in sterile Petri dishes (Fig. 1, C). The latter were placed immediately in desiccators of various relative humidities.

The desiccators were stored in constant temperature chambers at a wide range of temperatures. After storage intervals of 3, 10, 28, 51, 81, 109, 139, 173, 187 and 232 days 2 beads and 2 cover slips were removed from each desiccator. The beads and cover slips were partially imbedded in Nutramigen agar in Petri dishes and incubated at 22° C. If growth occurred, the conidia or ascospores were considered viable (Figs. 1, B and D). When negative results were obtained, the spores were retested for at least 3 subsequent storage periods.

The effect of wetting on longevity of the ascospores was studied at 24° and 31° C. Sterile glass beads were dipped in a water suspension of ascospores, air-dried, and placed in desiccators.

Relative humidities were regulated in desiccators with sulphuric acid solutions. The concentrations of acid necessary to give the different relative humidities at the different temperatures were calculated from the vapor pressures listed in the International Critical Tables (13). Since relative humidity over sulphuric acid will change by only about 2 per cent between 3° and 37° C., a concentration between that required for the 2 extremes was used at each of the temperatures for a specific relative humidity. For example, the percentage of acid required to give a relative humidity of 10 per cent is 65.2 at 37° and 64 at 3° C. In this experiment, 64.2 was used at all temperatures resulting in an actual relative humidity of 10.5 per cent at 37° and 8.0 per cent at 3° C.

EXPERIMENTAL RESULTS.—*Longevity of the endoconidia.*—The endoconidia remained viable longest under cool, dry conditions. In general, the lower the relative humidity and temperature, the greater the longevity (Table 1). The endoconidia were viable at 3° C. and 10 per cent relative humidity for 173 days, but were non-viable after 187 days under all conditions.

TABLE 1.—*The longevity of endoconidia of E. fagacearum in days at different temperatures and relative humidities*

Temperature °C.	Approximate percentage of relative humidity 95	75	50	25	10
37	<3	<3	3	10	10
31	3	3	3	10	10
24	10	10	81	109	139
18	10	28	51	139	139
15	10	28	81	139	139
12	10	51	139	139	139
9	28	139	139	173	173
6	139	139	139	173	173
3	51	139	173	173	173

TABLE 2.—*The longevity of ascospores of E. fagacearum in days[a] at different temperatures and relative humidities*

Temperature °C.	Approximate percentage of relative humidity 95	75	50	25	10
37	3	3	10	10	3
31	3	3	3	10	28
24	51	28	51	139	232
18	81	51	109	232	232
15	139	81	139	232	232
12	187	139	232	232	232
9	109	232	232	232	232
6	187	232	232	232	232
3	139	139	232	232	232

[a] A result of 232 days indicates the ascospores at that condition may be viable for longer periods since this was the last attempted isolation.

At 95 per cent relative humidity and 24° C. the endoconidia lived for 10 days or longer (no isolations were made between 10 and 18 days). Decreasing the temperature to 12° C. did not increase the longevity at 95 per cent relative humidity.

Longevity of the ascospores.—Relative humidity and temperature affected the longevity of the ascospores in a similar manner (Table 2). The ascospores were longer lived than the endoconidia. Ascospores were viable after 232 days at a large number of relative humidities at 18° C. and below.

While the ascospores lived longer than the endoconidia, the technique used with the 2 kinds of spores differed. The endoconidia were wetted and air-dried, whereas the ascospore masses were not. To check on this point ascospore masses were wetted and their longevity determined at 2 temperatures (Table 3). At 24° C. wetting reduced the longevity almost ½, yet at 31° C. wetting did not have this effect. The reason for this result is not readily apparent.

DISCUSSION.—Endoconidia and ascospores retained their viability longest at low humidities and low temperatures. This result agrees in general with the report of McLaughlin and True (7) for endoconidia of the oak wilt fungus. Similar results have been obtained by workers studying spore longevity of other fungi (1, 6, 9, 10). On the other hand a few fungi have been reported to live longest under cool, moist conditions (2, 3, 12).

Results of viability studies on glass beads and cover slips must be cautiously interpreted since this is not necessarily analagous to natural conditions. When

TABLE 3.—*The longevity of wetted and non-wetted ascospores of E. fagacearum in days at different temperatures and relative humidities*

Temperature °C.	Condition	Approximate Percentage of relative humidity 95	75	50	25	10
24	Wetted	14	14	21	91	42
	Not wetted	51	28	51	139	139
31	Wetted	10	10	10	28	28
	Not wetted	3	3	3	10	28

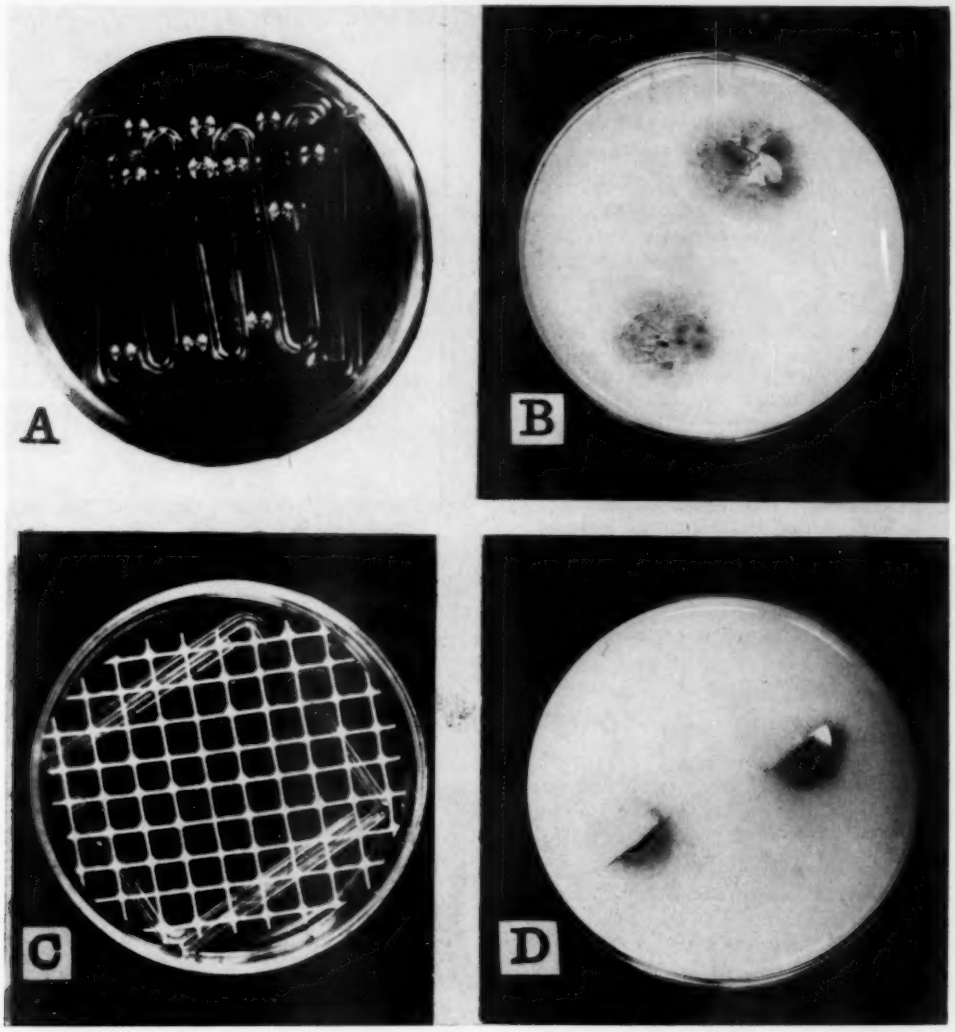

FIG. 1. A) glass beads on which endoconidia were stored. B) growth, on Nutramigen, of the oak wilt fungus from beads. C) cover slips on which ascospore masses were stored. D) growth, on Nutramigen, of the oak wilt fungus from cover slips.

the ascospores were separated from their mucilaginous matrix in water at 24° C. their longevity was decreased about ½.

Unfortunately, the method used herein did not lend itself to percentage germination observations. Nevertheless, these studies indicate that endoconidia and ascospores retain their viability long enough to be carried considerable distances by an unknown agent. The relative humidity in 1 day may vary from 95 to 25 per cent, and during spring and fall the temperature would not be greater than 24° C. (75° F.) for any great length of time. At these relative humidities and temperatures, the endoconidia remained viable for 10 and 109 days whereas the ascospores were viable for 51 and 139 days. It would therefore appear probable that ascospores would be more effective as inoculum than endoconidia, particularly since they are extruded from the perithecium in a mucilaginous matrix and would be expected to stick more effectively to the body of an insect or other carrier.

High relative humidities decreased the longevity of spores of *E. fagacearum*. This may be due to the effect of high relative humidity on the bound water-free water equilibrium within the spore's protoplasm affecting the metabolic activities (11). Since the free water in the spore is in equilibrium with the water surrounding the spore, a spore in a high relative humidity would have a greater percentage of water in the free state than one in a low relative humidity. Its metabolic rate would probably be greater with more free water present and the food reserves would be utilized faster. The spore would die sooner or would not have enough food reserve to germinate even if placed in optimum conditions for germination. Therefore, longevity in conditions of high humidity would be reduced. High temperatures may decrease

longevity by increasing metabolic activities, causing a quicker depletion of reserve foods resulting in death. Whatever the explanation may be, when the spores were stored under conditions of both high temperature and high relative humidity the effect was greatly enhanced and longevity was reduced more rapidly.

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A PHYTOPATHOLOGICAL NOTE

Resistance of bean leaf pulvini to fungi and viruses. C. E. YARWOOD. The relative freedom of Pinto bean pulvini to powdery mildew in greenhouse infections has been observed for several years. Other pathogens and other bean varieties were therefore also tested. The criterion of resistance was the lack of macroscopic growth of the pathogen with bean powdery mildew and rust, and the lack of formation of macroscopic lesions with anthracnose and the five viruses. Inoculations with powdery mildew (*Erysiphe polygoni* DC.), rust (*Uromyces phaseoli* (Pers.) Wint.), anthracnose (*Colletotrichum lindemuthianum* (Sacc. and Magn.) Briosi and Cav.), tobacco mosaic virus, tobacco necrosis virus, southern bean mosaic virus, and alfalfa mosaic virus on plants of the Pinto, Creaseback, and White Blue Lake varieties showed that the pulvini of these 3 varieties were resistant to these 7 pathogens, (Fig. 1) whereas the petioles and laminae of the same leaves were heavily infected. Only tobacco ring spot, of all pathogens tested, caused symptoms on the pulvinus as severely as on the petiole and lamina, and this was true with each of the same 3 bean varieties.

Uredospores of rust and conidia of anthracnose and powdery mildew were added separately to water extracts of pulvini, petioles, and laminae of Pinto bean. Germination occurred about equally well in all extracts.—University of California, Berkeley, California.

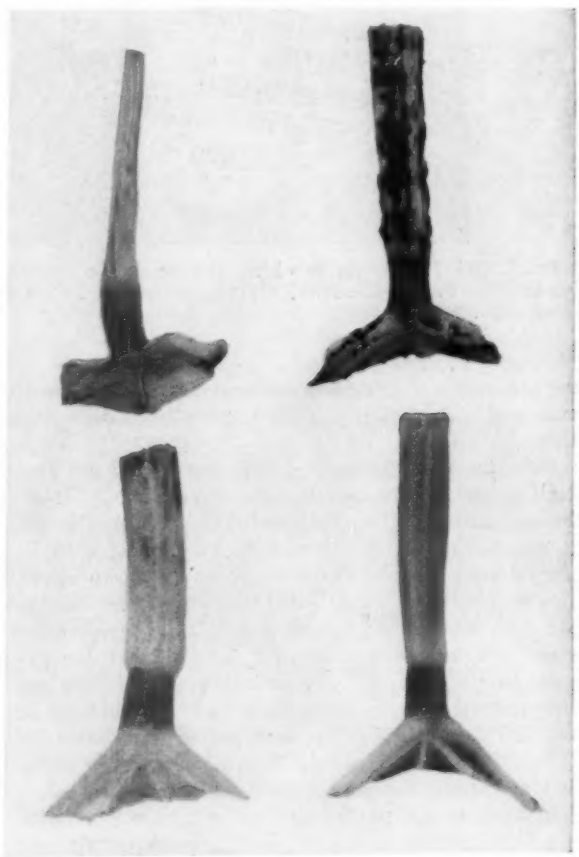

FIG. 1. Leaf pieces of Pinto bean. Upper left—healthy; upper right—infected with powdery mildew; lower left—infected with rust; lower right—infected with anthracnose. The pulvini are resistant to infection with these 3 fungi.

DISINFESTING ACTION OF WOOD PRESERVATIVES ON PLANT CONTAINERS [1]

Chester N. Roistacher and Kenneth F. Baker

SUMMARY

For 1 year after treatment with copper naphthenate, nursery flats remained self-disinfesting, and prevented carry-over of damping-off fungi from a planting of infested soil to one of pasteurized soil. Fungi were prevented from growing on and into the treated wood.

Root injury occurred to peppers in contact with the copper naphthenate-treated wood 1 year after application, even though the flats had been filled and dumped 6 times. This root damage was not reflected in leaf injury to pepper plants. Flats treated with copper naphthenate (2 and 3 per cent copper equivalent) and allowed to dry for 9–10 days after dipping, produced injury to seedlings of Iceland poppy, snapdragon, petunia, pansy, tobacco, Lobelia, Coreopsis, Nemesia, China aster, larkspur, and stock. Pepper, pea, nasturtium, and Calendula seedlings were not damaged. Typical root pruning was evident, however, on the plants from all treated flats. The root pruning and top injury were not due to the diluent, Stoddard Solvent.

Copper naphthenate should not be applied to containers used for seed sowing, for research on root or plant growth, or for growth of sensitive plants. It may be used effectively and safely as a disinfestant on glasshouse benches, even when plants are growing nearby. It may also be used when seedlings are planted 2–3 in. from the treated wood, and on benches or shelves used for potted plants.

Flats commercially treated with Wolman salts or Erdalith are neither self-disinfesting nor injurious to roots. Effective wood preservatives were thus shown to produce effects ranging from both self-disinfesting and root-injuring to those that are neither, because of complete fixation in the wood.

Wooden greenhouse flats may be treated with a persistent chemical to prevent pathogen carry-over from one planting to the next. Use of such self-disinfesting flats would eliminate the expense, labor, materials, and delayed planting involved in present treatment methods. Some progress toward a similar effect on fruit and vegetable picking boxes with a petroleum sulfonate preparation has been reported (8). A satisfactory flat disinfestant should kill the pathogen near or in contact with the wood, be nontoxic to living plant tissue, remain effective in the wood for long periods, and be relatively inexpensive. The results of studies on plants grown in wood flats treated with copper naphthenate, Wolman salts, and Erdalith are discussed in this paper.

COPPER NAPHTHENATE.—Copper naphthenate dissolved in light oil has been used increasingly during the last 20 years as a preservative for wood, fabrics, plastics, jute, plant containers, etc. Some commercial preparations of this chemical for treatment of plant containers have been advertised as nontoxic to roots and tops of plants, but paradoxically as also "intensifying root concentration," a condition said to be beneficial in transplanting. Several papers (1, 3, 7, 9) have also reported freedom from phytotoxicity, and only one (15) has considered it toxic to plants. Since this interpretation has not been confirmed by our tests, by experience of other plant pathologists, nor by evidence in commercial advertisements and correspondence, it seemed desirable to present our results.

Copper naphthenate, although relatively insoluble in water, slowly hydrolyzes to form copper hydroxide and free naphthenic acid, both actively toxic to wood-destroying fungi (10, 16). Bayley and Weatherburn (5) showed that copper naphthenate in fabric is partly converted to a benzene-insoluble form rather than lost during leaching or burial in soil. Once in soluble form, the copper may act on microorganisms, be leached out by water or adsorbed by the soil, or be precipitated in fungicidally inactive chemical combination (10).

Preventing disease carry-over.—The lasting quality and the disinfesting action of copper naphthenate in wooden flats were investigated. Redwood flats were dipped in a commercial preparation containing 3 per cent copper equivalent and allowed to dry for 10 days, a period greatly exceeding the recommended 24 hours. Five treated and 2 untreated flats were filled with soil naturally infested with *Rhizoctonia solani* K. and *Pythium* spp., while 2 treated and 2 untreated flats were filled with pasteurized soil as checks. The U. C. soil mix (6) was used throughout these and subsequent tests. It is improbable that the amount of peat in this mixture affected the action of the copper naphthenate, although peat mixtures of greater acidity are known (10) to induce leaching of this material. All flats were planted with hot-water treated seeds (4) of California Wonder Bell pepper in a well-ventilated greenhouse. Three weeks later, when seedlings in the checks were 2–3 in. high, there was severe damping-off in the infested soil. The flats were photographed, emptied, rinsed lightly with water, and all refilled immediately with pasteurized soil. Again they were planted with pepper seeds, and later observed for damping-off, which developed only around the margins of the untreated flats that had formerly contained infested soil (Fig. 1F). This cycle of following infested soil with pasteurized soil was repeated, so that in 4 months each flat had been filled and emptied 4 times.

Carry-over of pathogens by flats from infested to pasteurized soil was prevented by the copper naphthenate treatment. Even after 2 complete cycles, no damping-off was visible in the 5 treated flats filled with pasteurized soil, whereas the 2 comparable untreated flats invariably showed evidence of damping-off near

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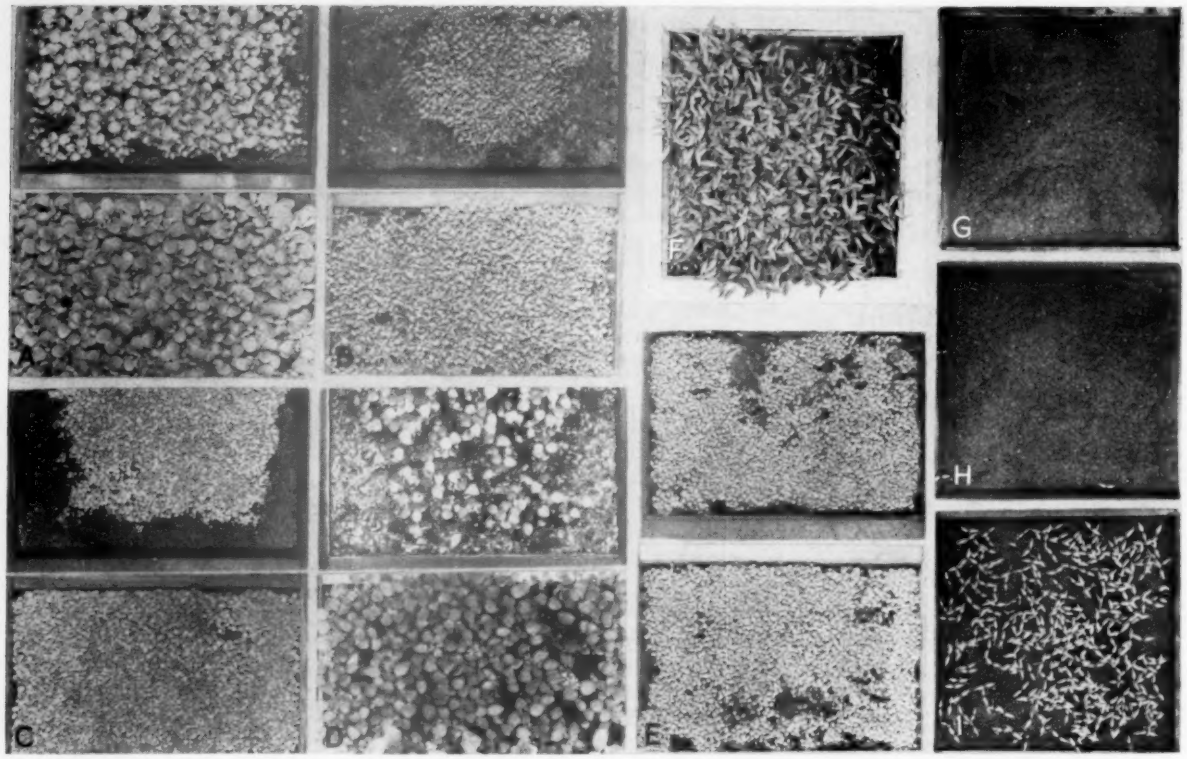

FIG. 1. Toxicity of copper naphthenate-treated flats to developing seedlings. A-E) Half-flats showing seedling growth in pasteurized soil in treated (upper) and untreated (lower) flat in each case. Flats planted with stock (A), Shirley poppy (B), tobacco (C), larkspur (D), and tobacco (E). Flats in A, B, and C treated with 3 per cent copper naphthenate 10 days before planting; flat in D treated with 2 per cent copper naphthenate 9 days before planting; flat in E dipped in diluent (Stoddard Solvent) 1 day before planting. F) Untreated flat filled with pasteurized soil following a previous planting with infested soil, showing damping-off of pepper seedlings near the margins from fungi carried over on flat. G) Absence of pepper seedlings in infested soil in untreated flat. H) Pepper seedlings in infested soil in treated flat, showing seedling emergence only in fungistatic zone near flat. I) Pepper seedlings in pasteurized soil in treated flat.

the margins (Fig. 1F). This was also true at the end of each subsequent cycle. The flats were washed and stored for 5 months and then refilled with infested soil as in previous tests. They were emptied 2 months later, lightly rinsed with water, immediately refilled with pasteurized soil, and planted. The last observations were made about a year after the initial treatment. While no damping-off was visible in any of the treated flats, it was prevalent in the untreated checks. The green color and characteristic odor of copper naphthenate were still present in the flats. When the soil was dumped, areas devoid of surface roots were noticeable where the copper naphthenate in the flat was still strong enough to kill root tips. However, roots had begun to cover the rest of the bottom, indicating that the toxic effects were greatly diminished.

In each of the treated flats containing infested soil pre-emergence damping-off was prevented in a ¾ in. margin. Copper naphthenate apparently restricted fungus growth but was relatively nontoxic to the pepper seedlings in this zone (Fig. 1H); the fungus killed all seedlings in the untreated flats (Fig. 1G). The perfect stand of seedlings in an untreated flat filled with pasteurized soil is shown in Figure 1I.

Root injury of pepper seedlings from the copper naphthenate in treated flats was clearly shown (Fig. 2A, C), even at the end of the fourth planting 4 months after the flats were dipped. Plants in the untreated flats showed no such injury (Fig. 2B, D).

Toxicity to seedlings.—Copper naphthenate apparently is relatively more toxic to damping-off fungi than to pepper seedlings (Fig. 1H). However, since some roots were injured, it was thought that smaller, more sensitive seedlings might be injured near the border of the flat. To test this, small redwood flats were dipped for 1 minute in a solution of 3 per cent copper naphthenate. The flats were allowed to dry 10 days in the open, after which they were filled with pasteurized soil and planted with seed of pepper, Calendula, stock, snapdragon, tobacco, Iceland poppy, Lobelia, Coreopsis, Nemesia, China aster, and larkspur. After 3 weeks, all seedlings except pepper and Calendula showed injury near the edges of the flats (upper half of Fig. 1A, B, C). Plants in the untreated flats showed no injury (lower half of Fig. 1A, B, C). The bottoms of the soil blocks from treated flats were free of roots, and root tips were blackened as in Figure 2A. However, the seedlings in the center of the flats usually

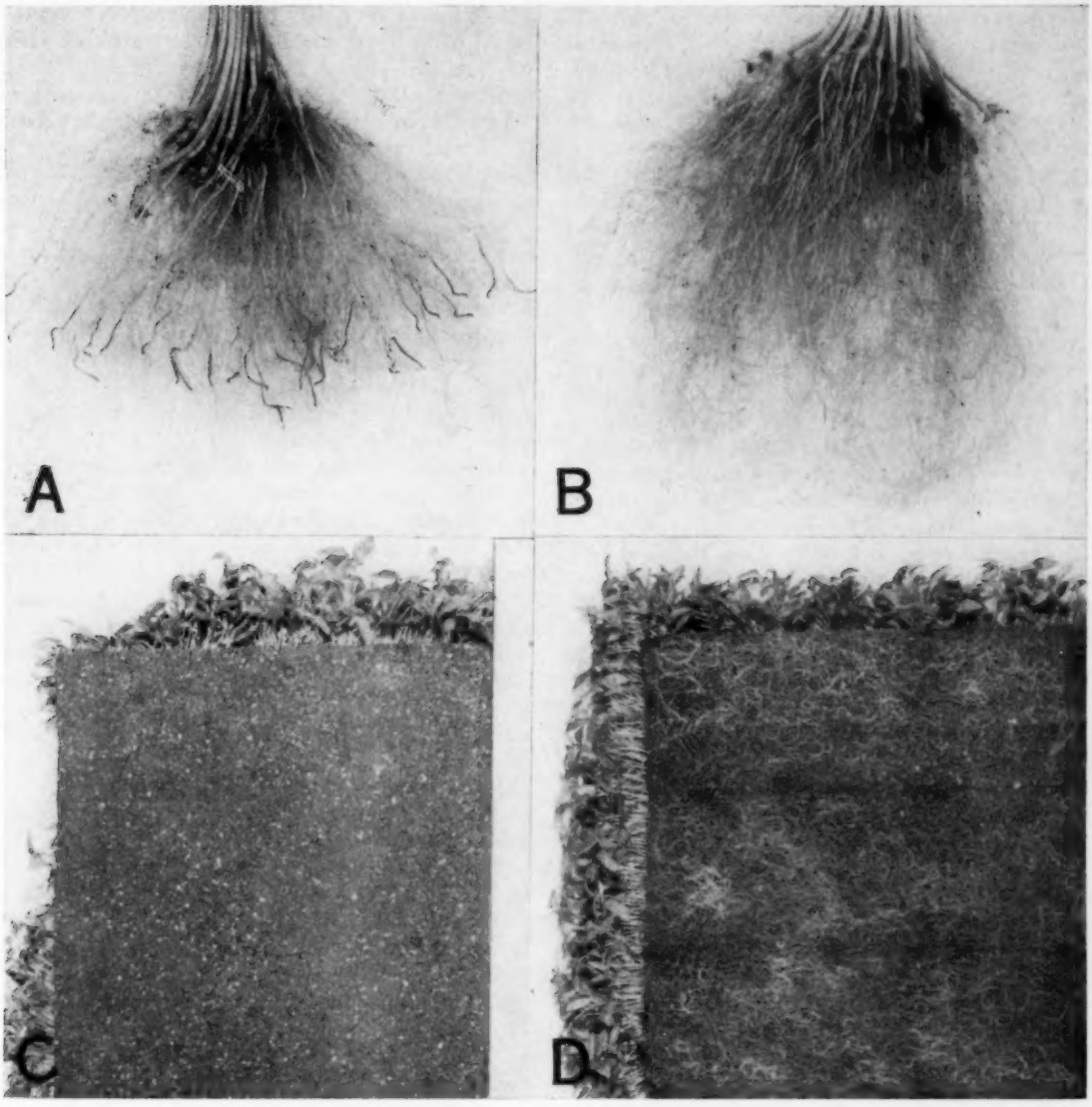

FIG. 2. Injury to roots of California Wonder Bell pepper from flats treated 4 months earlier with copper naphthenate. The soil in the flats had been changed 4 times, and plants grown continuously in them during this period. A) Roots of plants from flat C, showing blackened dead tips. B) Roots of plants from flat D, showing clean healthy tips. C) Bottom of soil from treated flat, showing absence of roots against the wood. D) Bottom of soil from untreated flat, showing abundant roots against the wood.

were not retarded in growth, even though the root tips were burned. Rapidly growing seedlings that soon reached the bottoms of the flats developed slight growth retardation (e.g., stock and larkspur, Fig. 1A, D; compare upper with lower half). Retardation and killing was general only along the border of soil adjoining the flat, and diffusion of toxic substances apparently extended as far as 2 in. from the edge of the wood.

This test was repeated, using 8 per cent copper naphthenate diluted with Stoddard Solvent to a 2 per cent copper-equivalent solution. After a 1-minute dip, the flats were dried for 9 days outdoors, and then planted with seed of pepper, pea, nasturtium, snapdragon, petunia, China aster, larkspur, pansy and stock. Untreated check flats were planted with an equal weight of seed. After 4 weeks all seedlings except pepper, nasturtium, and pea were markedly injured around the borders of treated flats (Fig. 1D). The injury was similar to that produced from the treated flats in the previous tests.

To determine whether the diluent was involved in the root injury a flat was dipped in undiluted Stoddard Solvent for 1 minute and dried for 24 hours. This flat and an untreated one were filled with pasteurized soil and seeded with Iceland poppy and tobacco. Since there was no root injury or marginal inhibition of seedling development (Fig. 1E), it is concluded that

the injury does not result from the diluent. This is corroborated by the fact that the fungicidal effect of copper naphthenate persists for a year, and that steaming of treated flats for several hours did not reduce either the fungicidal or phytotoxic effect, although the diluent would have been dissipated in each case.

There was no evidence from these or other tests that the naturally occurring decay-resisting materials in redwood have any toxic action on damping-off fungi on the wood (Fig. 1F) or on root development (Fig. 2D). Indeed, redwood flats are known to remain infested by damping-off fungi for several months.

OTHER MATERIALS.—It was evident from these tests that copper naphthenate diffuses from the wood and produces a zone in the contiguous soil that is inhibitory to both fungi and roots. This slow diffusion has also been noted by others (10, 11, 16). The possibility that other less diffusible preservative compounds might be equally effective against fungus carry-over without attendant root injury was investigated.

Flats were constructed from fir-shook that had been pressure-treated[2] with Wolman salts (Wolmanized) or with Erdalith. Wolman salts are mixtures of fluorides, phenols, and chromates; Erdalith contains arsenic, copper, and chromium (14). Both are effectively fixed in the wood. The flats were filled with infested soil and planted with peppers. Damping-off was not prevented near the margins in either case, as it had been with copper naphthenate. The soil was then dumped, the flats rinsed lightly with water, refilled with pasteurized soil, and planted with peppers. In all flats, damping-off developed around the margins, indicating that the fungi on the surface of the wood had survived. Roots in contact with the flats were not injured. It is concluded that treatment with Wolman salts or Erdalith does not produce a self-disinfesting surface but is noninjurious to plants or roots. These materials are commonly used for decay prevention in wood, and apparently are so highly fixed in the wood as to be safe for treatment of flats and benches to be used for growing plants. Disadvantages are high cost and lack of disinfestant action. Similar results have been reported (7) for Tanalith (a Wolman preparation) and Celcure (mixture of sodium dichromate, copper sulfate, chromic acetate, and dinitrophenol [14]). Copper 3-phenylsalicylate, reported (11) to be an effective fungicide with low toxicity to seedlings, was not tested because it is no longer available from the manufacturer.

DISCUSSION.—Copper naphthenate has an established place in the floriculture and nursery pharmacopoeia, despite the fact that it may be harmful to roots and to small seedlings. This material should not be used on seed flats or on containers in critical experiments. The injury caused to root tips would confuse inspection of nursery stock for the presence of root pathogens, and thus complicate plant certification. However, it can safely be used on flats or benches which are to be either filled with soil and used for large plants, or in which seedlings are planted 2 or 3 in. from the sides. It can safely be used also for benches, shelves, etc. on which potted plants are grown.

In commercial operations it is frequently necessary to disinfest a glasshouse bench before filling it with pasteurized soil. Formaldehyde (13) may be used if the house can be emptied of living plants. Where this is not possible, the bench may be painted with copper naphthenate with little danger to the surrounding plants if the ventilators are kept open until the solvent has evaporated. Sensitive plants such as maidenhair fern have been reported by growers to be injured by the solvent under conditions of inadequate ventilation. We have been informed by Dr. A. W. Dimock that copper naphthenate is used for such bench disinfestation by New York growers.

Treatment of flats with this material was found to render them self-disinfesting for a year. Where soil is pasteurized in continuous or bulk equipment rather than in containers, this feature of treated flats would be especially useful.

Although copper naphthenate is fairly expensive, growers have found that its use on pine or fir wood is economical from the standpoint of decay prevention alone. The self-disinfesting property is an added benefit without extra cost. Moreover, copper naphthenate purchased in bulk (8 per cent concentration) and diluted to 2 or 3 per cent with Stoddard Solvent is only about half as costly as in proprietary mixtures. The rusting of iron nails by the chemical (2, 12) may be avoided by the use of aluminum nails (2).

Some of the many available treating materials might be less firmly fixed in the wood than are Wolman salts or Erdalith, and prove lethal to fungi growing over the surface, although noninjurious to plant roots. Investigators of wood-decay preventives might profitably examine this possibility in the course of screening tests.

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[2] This material was treated by American Lumber and Treating Company, Los Angeles, California.

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ANTIBIOSIS IN RELATION TO PYTHIUM ROOT ROT OF SUGARCANE AND CORN [1]

Leander F. Johnson [2]

SUMMARY

A study was made of the effect of antagonistic soil microorganisms on Pythium root rot of sugarcane and corn. Soil microorganisms were isolated and tested for antibiosis to *Pythium arrhenomanes* Drech. Isolates of many different genera were found to produce antibiotics which inhibited the fungus. The zone of inhibition produced in agar cultures by certain of the antagonistic types persisted for more than 30 days.

Data obtained from greenhouse tests showed that certain antagonistic microorganisms, when placed in infested soil which had been previously sterilized, significantly controlled Pythium root rot of corn. Better control was obtained by the use of actinomycetes than with bacteria or fungi. There was a general correlation between the magnitude of the zone of inhibition produced by the actinomycetes and their ability to control root rot, better control being produced by isolates which produced large zones of inhibition. In general, better control was obtained with isolates whose zones of inhibition persisted for more than 10 days than with isolates whose zones were overgrown before the eleventh day.

Attempts to control Pythium root rot by the use of microorganisms not antagonistic to the root rotting fungus showed that none of the isolates tested consistently controlled the disease.

The results tend to emphasize the importance of antagonistic microorganisms, especially actinomycetes, as a factor affecting the severity of Pythium root rot.

The study of microorganisms antagonistic to *Pythium arrhenomanes* Drechsler, which causes a root rot of sugarcane and corn, had its beginning with the discovery by Tims (9), in 1932, of several actinomycete isolates which inhibited the growth of this pathogen in artificial culture. One of these actinomycetes, incorporated into sterilized soil which had been infested with *Pythium*, reduced the severity of root rot. LeBeau (5), in 1939, found isolates of *Trichoderma* in Louisiana soils which were antagonistic to the root parasite. These findings and the fact that sugarcane root rot is much more severe in some Louisiana soil types than in others (8) led to the hypothesis that these differences in disease severity might be due to differences in populations of soil microorganisms antagonistic to the root rot pathogen. Results of surveys of Louisiana sugarcane soils by Cooper and Chilton (3), Luke (6), and Connell (1) indicated that 23 per cent of the actinomycetes, 16 per cent of the fungi, and 3 per cent of the bacteria isolated from Louisiana soils were antagonistic to *P. arrhenomanes* in artificial culture. The antibiotic activity of the actinomycetes, estimated from the numbers found and their average inhibition, was higher in the light (non-root rot) soils than in the heavy (root rot) soils.

These results indicated that antagonistic soil microorganisms, especially actinomycetes, might be a factor affecting the severity of Pythium root rot of sugarcane. However, to arrive at a more satisfactory estimation of the antibiotic activity of the microorganisms present in Louisiana soils, more specific data were needed concerning their effect upon Pythium root rot in infested soil. The objectives of the present investigation were to determine the effect of microorganisms, antagonistic to *P. arrhenomanes* in agar cultures, on the severity of Pythium root rot under greenhouse conditions, to study nonantagonistic microorganisms in a similar manner, and to evaluate the results in terms of the type of organism, the degree of antagonism, and the type of antagonism exhibited.

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The writer wishes to express his sincere appreciation to Dr. H. E. Wheeler and Dr. S. J. P. Chilton for their many suggestions in the various phases of this study.

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MATERIALS AND METHODS.—The dilution technique for isolating soil microorganisms was patterned along standard lines (3). The usual final dilution of soil (on a dry soil basis) to water, and the agar medium used for each type of microorganism studied were as follows:

Fungi	1:10,000	Soil extract glucose nitrate agar (plus Rose Bengal)
Bacteria	1:10,000,000	Thornton's mannitol asparagin agar
Actinomycetes	1:100,000	paraginate agar

Microorganisms selected from the dilution cultures were tested for antibiosis to *P. arrhenomanes* on Czapek's sucrose nitrate agar in Petri dishes by using the streak method. Disks of *Pythium* were placed opposite the 48-hour old streaks of the organisms to be tested. Five days later the clear zone of inhibition, if any, between the 2 organisms, was measured in mm. In order to determine how long the clear zone persisted, measurements of the radius of the antagonist and the clear zone were made for a period of 25 days thereafter. All tests were performed in triplicate.

In obtaining the isolates to be tested for antagonism to *Pythium arrhenomanes*, an attempt was made to select as many different cultural types as possible. After several hundred isolates had been tested, antagonistic types were selected for further study. The selected fungal isolates were tentatively identified to genera, but no attempt was made to identify the bacterial and actinomycete isolates. Several distinct cultural types of bacteria were encountered which indicated that the bacterial isolates represented several different genera. Preliminary observations of the actinomycete isolates indicated that most, if not all, belonged to the genus *Streptomyces*, and that many different species were represented.

In order to determine the ability of the isolates to control Pythium root rot, 4-day-old Czapek's agar cultures of *P. arrhenomanes*, cut into blocks approximately 1.0 cm. square, were stirred into 3 in. pots of soil which had been previously sterilized. The isolates to be tested were grown on Czapek's agar for 7 days, cut into blocks, and then stirred into the soil immediately after infestation with *Pythium*. One-third of a Petri dish culture of *Pythium* and of the isolate to be tested were added to each pot of soil. Three grains of White Tux-Pan corn were planted in each pot 1 week after infestation. After 2 weeks of growth, the roots were washed free of soil and the severity of root rot was determined by rating the plants in visual infection classes (Fig. 1).

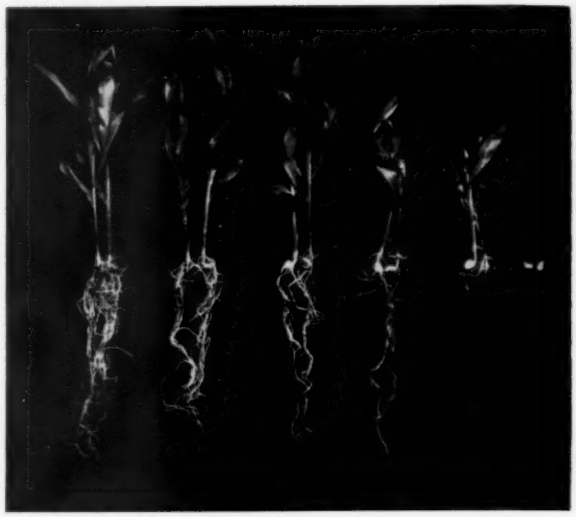

FIG. 1. *Pythium arrhenomanes* infection classes on corn roots. Left to right: 0, no infection; 1, trace; 2, slight; 3, moderate; 4, severe; 5, very severe.

EXPERIMENTAL RESULTS—A list of the types of microorganisms selected and their average inhibition in agar cultures is presented in Table 1. Species of *Penicillium*, *Spicaria*, and *Aspergillus* were the most common soil fungi found to inhibit *P. arrhenomanes*. One Penicillium isolate produced a wider zone (42 mm.) than did any other microorganism tested. The average clear zone produced by the actinomycetes was wider than that produced by the fungi, but the latter were more inhibitory than the bacteria.

TABLE 1.—*Inhibition of Pythium arrhenomanes in agar cultures by selected soil microorganisms. Measurements in mm.*

Organism type	Number selected	Radius of antagonist		Clear zone		Total inhibition (radius + clear zone)	
		Range	Ave.	Range	Ave.	Range	Ave.
Fungi	58	2-19	8.9	1-42	7.4	3-50	16.3
Penicillium	17	2-10	7.7	1-42	10.1	3-50	17.8
Spicaria	13	8-14	10.2	1-15	8.2	9-23	18.5
Aspergillus	9	6-17	10.8	1-19	5.1	7-28	15.9
Monosporium	3	6- 8	7.0	2- 4	3.0	8-12	10.0
Gliobotrys	2	7- 9	8.0	20-21	20.5	28-29	28.5
Monilia	2	2- 3	2.5	13-15	14.0	16-17	16.5
Hormodendrum	1		6.0		1.0		7.0
Botrytis	1		7.0		1.0		8.0
Fusarium	1		5.0		13.0		18.0
Undetermined	9	5-19	10.3	1- 2	1.3	7-21	11.7
Bacteria	55	2-24	7.4	1- 8	2.1	3-25	9.5
Actinomycetes	58	2- 6	3.8	1-31	9.6	4-34	13.4

The persistence of the clear zone was quite variable with the fungal and actinomycete isolates, lasting, in many cases, for more than 30 days after inoculation. With a few exceptions, *Pythium* was completely inhibited after the seventh day by the fungi, and any overgrowth of the zone was usually from the antagonistic isolate. However, in many cases, *Pythium* grew to some extent over the zone produced by the actinomycete isolates. Most of the isolates of certain genera, such as *Monilia* and *Spicaria*, had a tendency to produce a stable zone. On the other hand, none of the *Aspergillus* and few of the *Penicillium* isolates produced a zone which persisted for more than 15 days.

In order to determine their ability to control Pythium root rot, all the isolates listed in Table 1 were incorporated into soil which had been previously sterilized and infested with *Pythium*. The severity of root rot of corn grown in these soils was calculated. The microorganisms were tested in groups of from 9 to 12 in 5 replicates. Two types of controls were used: soil infested with *P. arrhenomanes* only, and soil which received no treatment other than sterilization. An analysis of variance was made for each group, and the least significant difference at the 5 per cent level of probability was calculated.

Most of the isolates produced some control of Pythium root rot, but in many cases, the reduction was not statistically significant. Twenty-one per cent of the fungi, 20 per cent of the bacteria, and 34 per cent of the actinomycetes significantly reduced the severity of the disease. The control produced by actinomycete isolate AA-5 is shown in Fig. 2. A large amount of variation among replicates was noted in the tests, and for this reason, it seemed desirable to determine if the isolates were consistent in their ability to control the disease. Therefore, all the isolates which controlled root rot and some selected isolates which did not were retested twice. The "degree of control" of each isolate is a numerical value obtained by subtracting the average degree of infection (Fig. 1) from the infested control. The degree of control of the isolates tested 3 times is an average of the 3 values obtained.

FIG. 2. The reduction of Pythium root rot of corn by an actinomycete isolate added to sterilized, artificially infested soil. Left to right: infested control, isolate AA-5, and non-infested control.

The numbers of microorganisms which controlled the disease and the average degree of control of each organism type are in Table 2. Some of the isolates

TABLE 2.—*The numbers of microorganisms which controlled Pythium root rot of corn under greenhouse conditions and the degree of control obtained*

Organism type	No. tested	No. producing control [a]	No. retested	Type of control [b]				Ave. degree of control
				none	poor	moderate	good	
Fungi	58	12	24	8	4	9	3	.25
Penicillium	17	3	6	3	0	2	1	.30
Spicaria	13	4	8	3	1	3	1	.40
Aspergillus	9	1	3	0	1	1	1	.24
Monosporium	3	0	1	1	0	0	0	.22
Gliobotrys	2	0	1	1	0	0	0	—.05
Monilia	2	1	1	0	0	1	0	.13
Hormodendrum	1	0	0	..	..	..	..	—.54
Botrytis	1	0	0	..	..	..	..	.18
Fusarium	1	0	1	0	1	0	0	.13
Undetermined	9	3	3	0	1	2	0	.17
Bacteria	55	11	26	8	11	7	0	.29
Actinomycetes	58	20	29	6	10	6	7	.46

[a] Significantly different from the infested control at the 5 per cent level of probability.

[b] good—significant control obtained in all 3 tests.
moderate—significant control obtained in 2 of the 3 tests.
poor—significant control obtained in only 1 of the 3 tests.
none—no significant control obtained in any of the 3 tests.

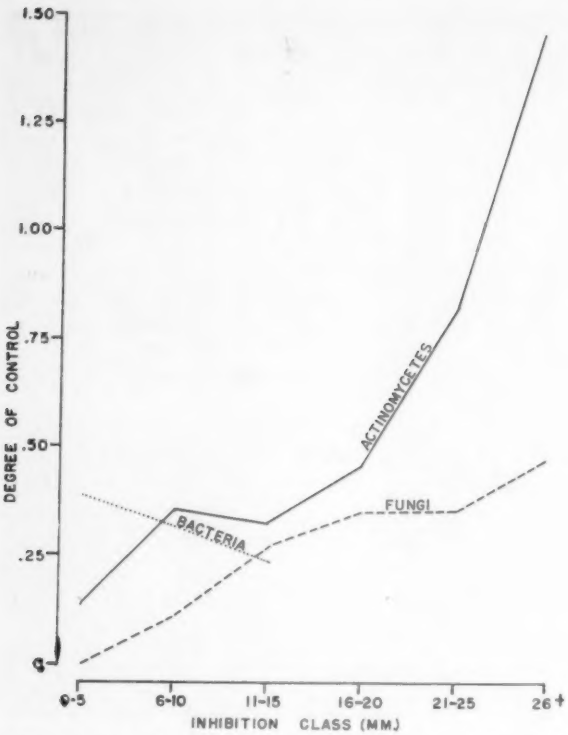

FIG. 3. The relation between the magnitude of the total inhibition produced by isolates antagonistic to *Pythium arrhenomanes* and their ability to control Pythium root rot of corn.

were constant in their ability to control the disease, while others behaved erratically in this respect. More actinomycete isolates produced good control than did the fungi, while none of the bacteria produced significant control in all 3 tests. The only genera of fungi which produced control in all 3 cases were isolates of *Penicillium*, *Aspergillus*, and *Spicaria*. As a group, the actinomycetes produced a higher degree of control than did the fungi or bacteria.

It seemed desirable to determine the relationship between the degree of control produced by the isolates and their antagonistic activity in agar cultures. The antagonistic isolates were placed in classes according to the magnitude of their total inhibition, and the average degree of control obtained with the isolates in each inhibition class was calculated. The results are graphically illustrated in Fig. 3. The data indicate that, in general, more control was produced by the actinomycete isolates than by the fungal or bacterial isolates. The fungi, and especially the actinomycetes, which produced large zones of inhibition, gave more control of the disease than did isolates which produced smaller zones of inhibition.

The antagonistic isolates of fungi and actinomycetes were divided into 3 groups, according to the length of time the zone of inhibition persisted. The average degree of control produced by each of these groups was calculated. The relation between the persistence of the zone of inhibition and the degree of control produced by these microorganisms is shown in Fig. 4. The data indicate again, that the actinomycetes produced more control than did the fungi. The data also indicate that, in general, better control was obtained with isolates producing zones of inhibition which persisted for more than 10 days than with isolates producing zones which were overgrown before the tenth day.

No attempts have been made in the past to determine whether or not soil microorganisms which are not antagonistic to *Pythium* in agar cultures will control the root rot disease under greenhouse conditions. Therefore, an attempt was made in this study to determine whether or not control could be obtained by the use of nonantagonistic microorganisms isolated from Louisiana soils.

A total of 181 nonantagonistic isolates were tested in the greenhouse in the manner previously described. These isolates consisted of more than 14 genera of fungi and several distinct cultural types of bacteria and actinomycetes. All isolates which produced some control were retested twice. These repeated tests showed that 7 isolates produced significant control of the disease in 1 of the 3 tests, while only 1 isolate produced control in 2 of the 3 tests. None of the isolates produced control in all 3 tests. These results suggest that microorganisms which are not antagonistic to *Pythium arrhenomanes* in agar cultures are not of major importance in reducing the severity of Pythium root rot.

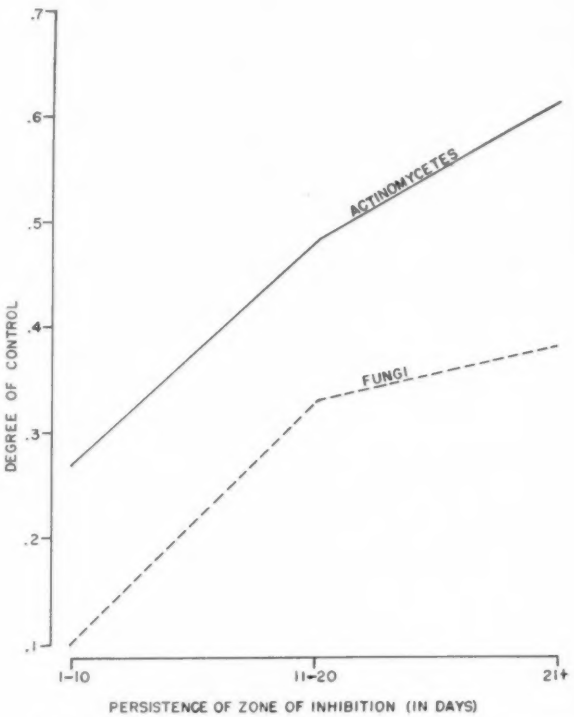

FIG. 4. The relation between the persistence of the zone of inhibition produced by isolates antagonistic to *Pythium arrhenomanes* and their ability to control Pythium root rot of corn.

DISCUSSION.—Although some isolates which are antagonistic to *Pythium arrhenomanes* in agar cultures do not control Pythium root rot in the soil, there exists a positive correlation between the 2 phenomena. This is evident not only from the correlation between high antibiotic activity in agar cultures and high degree of control of the disease, but also from the fact that none of the nonantagonists consistently reduced disease severity. Therefore the results of this study tend to validate the use of surveys (1, 3, 6) as a means of estimating the antibiotic potential of soils.

This study also suggests that antagonistic actinomycetes play a larger role in reducing root rot severity than fungi or bacteria. This is in agreement with the results of a previous study (4) in which sterilized soil infested with *Pythium* was allowed to become recontaminated by exposure to the atmosphere. In such soil, the severity of root rot progressively decreased, and this decrease was accompanied by and generally correlated with an increase in the antibiotic activity of the actinomycetes. No such correlation was found in regard to the bacteria or fungi.

Because of the large number of different types of microorganisms which are antagonistic to *P. arrhenomanes* it appears that several different antibiotics are involved. The fact that some antagonistic types controlled the disease and others did not, furnishes further evidence for this belief. It is also probable that among the isolates which consistently reduced the severity of the disease more than 1 antibiotic was active, since it seems unlikely that isolates of *Penicillium, Aspergillus, Spicaria,* and several cultural types of *Streptomyces* produced the same antibiotic. The data indicate that these microorganisms produced antibiotics which were a) produced in the soil, b) not inactivated by soil colloids, c) not readily broken down by the chemical constituents of the soil, and d) not immediately broken down by the returning soil microflora.

To simulate natural conditions more closely, it would have been desirable to test antagonists in nonsterilized soil. This was not done because it has not been possible to obtain severe root rot in nonsterilized soil infested with *Pythium.*

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HOST NUTRITION IN RELATION TO MULTIPLICATION OF TOBACCO MOSAIC VIRUS IN TOBACCO [1]

Lewis G. Weathers [2] and Glenn S. Pound

SUMMARY

The relation of nitrogen, phosphorus, potassium, and concentration of balanced solutions to host growth and concentration of tobacco mosaic virus (tobacco virus 1) in *Nicotiana tabacum* was studied by supplying the host with nutrient solutions varying in nutrients and nutritional levels. Virus concentration was measured by local lesion inoculation to the F_1 hybrid of *N. tabacum* × *N. glutinosa* and by ultraviolet absorption (optical density) of purified virus suspensions.

With nitrogen levels ranging from 21 to 1050 p.p.m. the virus concentration as measured by local lesion assays of untreated expressed juice from systemically-infected tip leaves was inversely correlated with the increase of nitrogen. With purified virus preparations taken from whole plants the highest virus concentration as measured both by optical density and lesion counts was found to be directly correlated with the amount of nitrogen supplied to the host. Optimum host growth was at 630 p.p.m. This discrepancy was shown to be due to the fact that increase in nitrogen also increased the amount of an inhibitor present in the plants, which reduced the virus activity of the expressed juice.

With phosphorus there was a growth gradient increasing from 3 p.p.m. up to 237 p.p.m. Above 237 p.p.m. growth decreased. In both lesion and optical density assays the phosphorus levels that effected stunting did not decrease the virus concentration. The virus concentration at 547 p.p.m. was equal to or higher than the virus concentration at 237 p.p.m. where maximum growth occurred.

With variations in the concentration of balanced solutions there was a close correlation of host growth and virus concentration. The highest lesion counts and optical density readings were at the 1H level where maximum growth was recorded.

Variations in potassium had little effect on plant growth and likewise little effect on virus concentration. Variations in lesion counts and ultraviolet absorption (optical density) were directly related to variations in growth although actual differences were considered too slight to be significant.

In a preceding paper (5) it was shown that the multiplication of turnip virus 1 in *Nicotiana glutinosa* L. and *N. multivalvis* Lindl., as measured by virus activity of expressed sap, was markedly affected by changes in nutrition of the host. The effects of varying amounts of nitrogen and potassium and the concentration of balanced solutions on virus multiplication coincided with corresponding effects of those nutrients on plant growth. In the case of phosphorus, virus concentration correlated directly with the amount of phosphorus supplied to the host and was independent of host growth. These results were in very close agreement with the studies of Cheo et al. (2) with cucumber virus 1 in spinach. A survey of the literature indicates that these 2 investigations are the only controlled studies thus far conducted that have been concerned with the effect of host nutrition on multiplication of viruses other than tobacco mosaic virus.

Previous studies concerning host nutrition and virus activity have been primarily concerned with the manner in which nitrogen affected the multiplication of tobacco mosaic virus. Results of these investigations have been contradictory. The investigations herein reported were conducted, therefore, to determine to what extent the addition of different amounts of nitrogen, phosphorus, potassium, and balanced solutions affected host growth and multiplication of tobacco mosaic virus in tobacco.

MATERIALS AND METHODS.—*Nicotiana tabacum* L. var. Connecticut Havana no. 38 was the host plant used for systemic multiplication of the virus. Methods of growing plants, inoculations, and the experimental design of the assay were identical to those described in previous papers (4, 5).

Local lesion assays of virus concentration were made by half-leaf inoculations on plants of the F_1 hybrid of *Nicotiana tabacum* × *N. glutinosa*. Inocula were applied with cheese cloth pads. Differences in comparisons of any 2 levels were tested for significance by analysis of variance. For purposes of simplification, however, overall averages of the 9 replications of each treatment are given. These treatment averages do not always accurately indicate the difference between treatments, due to variations between leaves of the assay plants. All comparisons were statistically significant except where otherwise indicated. The effect of the nutrients on host growth was measured by harvesting the uninoculated series of plants and recording fresh weights.

The local lesion method for determining virus concentration is based on infectivity and as such is only a measure of infective virus. Where noninfective virus is present, it is apparent that a true measure of the virus concentration is not provided by this method. Furthermore, if aggregation of virus particles has been brought about, or if inhibitors of infectivity are present, the local lesion count will be lower than the actual concentration (10). Because of these limitations of the local lesion method, the concentration of the virus was also measured by ultraviolet absorption

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(11).

The procedure used for preparing the virus for determining concentration by ultraviolet absorption (optical density) was as follows. Twenty to 40 days after inoculation infected plants were cut at the soil level and weighed to record the effects of the treatments on plant growth. The plants were chopped into very small pieces, thoroughly mixed, and a representative sample, usually a weight equivalent to the total weight of the treatment showing the smallest growth, was removed and frozen for 24 hours. The frozen samples were triturated in 0.1M phosphate buffer, pH 7, in a Waring blendor until the preparations were homogeneous. The mixtures were expressed through 2 layers of cheese cloth. An aliquot (usually 30 c.c.), after heat clarification, was subjected to 2 cycles of low and high speed centrifugation. The final pellet was resuspended in buffer and the ultraviolet absorption (optical density) of each purified fraction was determined by means of a Beckman model DU spectrophotometer set for a wave length of 265 mu. In certain cases the optical density of the purified fractions could not be determined due to the high concentration of virus and it was found necessary to dilute the fractions with buffer.

Takahashi (11) has shown that the optical density of a solution of purified tobacco mosaic virus is proportional to the virus concentration. In 3 preliminary trials, known dilutions of purified virus gave optical density readings inversely proportional to dilution of the fractions, thus confirming Takahashi's results. There was a geometric gradient of optical density that corresponded exactly with the geometric dilution gradient of the virus solution. This indicated that the method was very reliable.

The residue of the first low speed centrifugation was tested for virus by inoculating the local lesion host with the residue. It was found that very little virus was lost. Lesion counts of purified suspensions compared, by half leaf inoculations, to lesion counts of clarified sap at similar dilutions indicated that recovery of the virus was almost complete.

EXPERIMENTAL RESULTS.—*Nitrogen.*—In all studies, tobacco plants were grown in 21, 210, 630 and 1050 p.p.m. of nitrogen. Infected plants showed a growth response similar to that of noninfected plants at the various nitrogen levels (Tables 1 and 2). Plants which received 21 p.p.m. and 1050 p.p.m. of nitrogen made the least growth. Plants at 21 p.p.m. were very chlorotic while those at 1050 p.p.m. were very dark green. Plants at 210 and 630 p.p.m. made the best growth.

Systemic symptoms appeared at all nutrient levels at approximately the same time. Over a period of 3 to 4 weeks, however, the symptom expression varied markedly with the treatment. Tip leaves from infected tobacco plants from the 4 levels of nitrogen are shown in Fig. 1. A distinct gradient of symptom severity existed, increasing from the lowest to the highest nitrogen level. At 21 p.p.m. the mottling was very diffuse. As the nitrogen increased the mosaic pattern became more prominent and individual chlorotic areas were more pronounced. At the highest level of nitrogen there were many large islands of darker tissue and the leaves were noticeably more distorted and crinkled.

Results of 2 experiments using local lesion assays of crude sap are in Table 1. Assays of inoculated leaves were made at 2 and 4 days after inoculation. Assays from tip leaves were made at various intervals following the first appearance of symptoms. Regardless of the source of the assayed tissue, virus concentration failed to coincide with the growth curve of the plants, symptom severity, or with increasing amounts of nitrogen supplied to the host. In all cases the virus concentration of plants at the nitrogen-deficient level was about as high or higher than the concentration at any other nitrogen level. Virus concentration curves were similar in all assays ranging from 2 days after inoculation through 23 days, indicating that the virus response to the various nitrogen treatments was not a result of variation in the rate of virus multiplication.

TABLE 1.—*The effect of nitrogen nutrition on the multiplication of tobacco mosaic virus in tobacco as measured by infectivity of expressed juice*

Nitrogen levels (p.p.m.)	Average number of lesions per half leaf of assay plant at indicated intervals after inoculation of *N. tabacum* plants [a]				Average fresh weight of healthy plants (gm.)
Experiment 1	2 days [b]	5 days	9 days	19 days	
21	252.6	178.6	152.3	192.3	8.5 [c]
210	230.3	153.9	163.3	184.5	11.3
630	223.3	149.2	142.0	160.7	12.9
1050	112.0	110.0	109.2	131.1	10.6
Experiment 2	2 days [b]	4 days [b]	11 days	23 days	
21	271.4	193.5	172.6	170.6	6.4 [d]
210	293.4	194.2	163.7	182.4	9.7
630	212.7	151.0	148.9	183.5	11.5
1050	178.3	139.4	140.2	180.2	9.0

[a] Values are averages of 9 replications.
[b] Assays of inoculated leaves. All other assays are of systemically-infected leaves.
[c] Measured on 9th day assay.
[d] Measured on 11th day assay.

TABLE 2.—*The effect of nitrogen nutrition on the concentration of tobacco mosaic virus in tobacco*

Nitrogen levels (p.p.m.) and assay intervals	Average fresh weight per plant (gm.)	Virus activity of clarified juice[a]	Virus activity of purified fractions[a]	Optical density of purified fractions[b]
29 days[c]				
21	6.1	184.2	121.7	0.604
210	8.7	192.1	120.3	0.648
630	10.2	203.4	180.5	0.702
1050	6.6	201.6	223.4	1.050
34 days				
21	6.3	143.6	159.2	0.613
210	11.1	181.7[d]	183.4	0.768
630	9.9	168.4	199.9	0.802
1050	7.8	172.2	196.8	0.860
24 days				
21	4.9	151.2	114.3	0.473
210	9.8	193.6	141.7	0.572
630	9.0	214.5	184.4	0.633
1050	9.1	249.6	216.3	1.000

[a] Virus activity refers to infectivity as measured by the local lesion reaction. Values shown are averages of 9 replications.
[b] Ultraviolet wave length of 265 mμ.
[c] Days after inoculation. Each assay was of a separate experiment.
[d] In half leaf comparisons 210 p.p.m. was consistently lower than 630 and 1050 p.p.m. See page 76 for an explanation of the failure of these figures to reflect differences accurately.

Since these results were not in agreement with results of other workers or with previous work with turnip virus 1 (5) many confirmatory experiments (not reported herein) were performed and similar results were obtained in every case.

Results (Table 2) of the lesion assays with heat-clarified and with purified preparations from whole plants showed that virus concentration coincided closely with the amount of nitrogen supplied to the host and not with the vegetative growth of the plants, in contrast to results with turnip virus 1. It is also significant that the results in these experiments were the reverse of data with lesion assays of crude sap (Table 1). The highest lesion count with both clarified juice and purified virus suspensions from whole plants occurred, in most cases, at 1050 p.p.m. and the lowest lesion count at 21 p.p.m. The optical densities of the purified virus fractions likewise coincided with the amount of nitrogen supplied to the host. The optical density of virus fractions from plants at 1050 p.p.m. was in all cases higher and those from plants grown at 21 p.p.m. lower than at other levels studied. The virus concentration curves from purified preparations as determined by both ultraviolet absorption and lesion counts were similar in all harvests.

Preliminary investigations were made to explain the discrepancy between the concentration curve of assays of crude extracts (Table 1) and the purified and partially-purified extracts (Table 2). As a possible explanation the presence of an inhibitor was investigated. Extracts from healthy plants growing at the 4 levels of nitrogen were prepared and mixed with a partially-purified extract of tobacco mosaic virus. The healthy extracts at dilutions of 1:50, 1:500, and 1:5,000 were added in the proportion of 1:1 to the virus extract at a dilution of 1:5,000. Thus, the final dilutions of the healthy extracts were 1:100, 1:1,000, and 1:10,000 and that of the virus 1:10,000. The various preparations at any one dilution level were compared in all possible combinations by half-leaf inoculations to the local lesion assay plants. In Table 3 are shown the average lesions per half leaf of each preparation in 2 separate trials. Four nitrogen levels were used in experiment 1 and 3 levels in experiment 2. In both experiments the highest lesion count occurred at the 21 p.p.m. nitrogen level and the lowest lesion count at 1050 p.p.m. Although inhibition was indicated at all dilutions, it was most marked at 1:100. From these preliminary investigations it appears that an inhibitor was present in the tobacco plants receiving supplements of nitrogen, and that increases in nitrogen increased the amount of inhibitor present. This would account for the higher lesion counts at the lower nitrogen levels, and vice versa. During clarification and purification of the virus for concentration determinations with the spectrophotometer the inhibitor was probably removed or inactivated. With the elimina-

TABLE 3.—*Effect of mixing extracts from healthy tobacco plants grown under different levels of nitrogen with a standard inoculum of tobacco mosaic virus*

Experiment	Average number of lesions per half leaf with standard inoculum diluted with extracts from healthy tobacco plants grown at—			
	21 p.p.m.	210 p.p.m.	630 p.p.m.	1050 p.p.m.
1[a]	103	72	64	37
2[b]	165	—	124	81

[a] Values shown are averages of 9 replications; healthy extract diluted 1:100.
[b] Values shown are averages of 6 replications; healthy extract diluted 1:100.

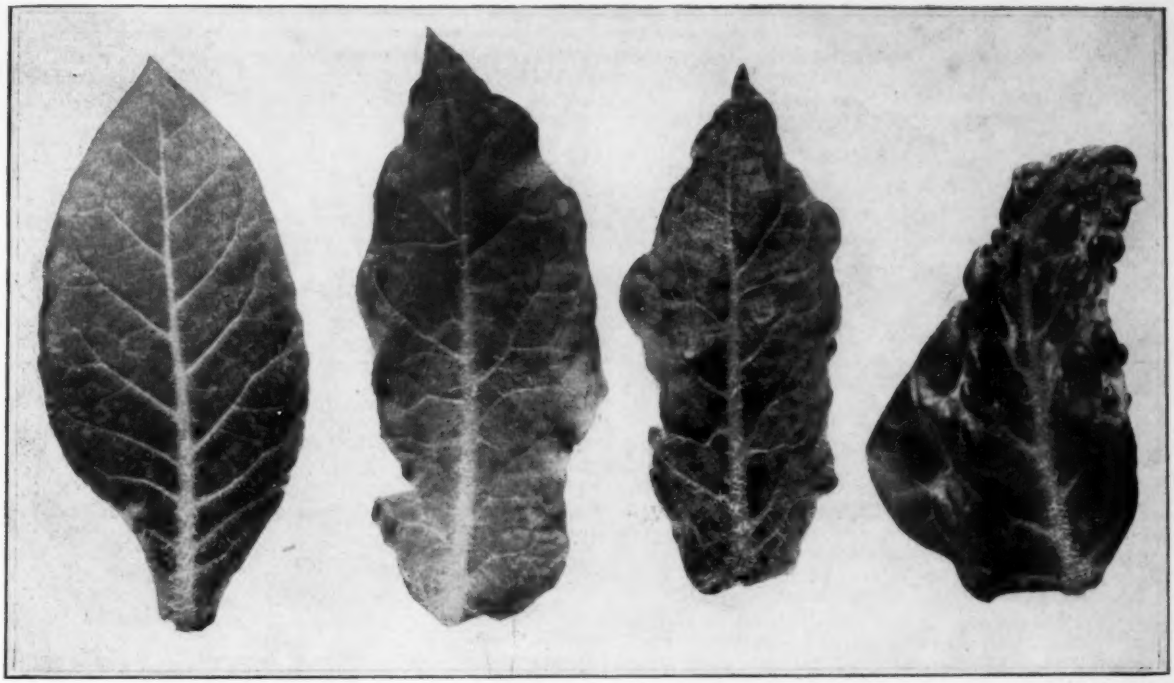

FIG. 1.—Systemic symptoms produced by tobacco mosaic virus on *Nicotiana tabacum* L. 30 days after inoculation at (left to right) 21, 210, 630 and 1050 p.p.m. of nitrogen.

tion of the action of the inhibitor the true relation of nitrogen to virus concentration was determined. Further investigations involving the presence of the inhibitor are underway.

Phosphorus.—Four levels of phosphorus (3, 93, 237 and 547 p.p.m.) were used in all experiments. Fresh weights of healthy plants were recorded at the time of the first assay. Plants grown at 3 p.p.m. were very much smaller than at other phosphorus levels, were darker green, and showed purple pigment indicative of phosphorus deficiency. The maximum growth was at 237 p.p.m. At 547 p.p.m. the plants were severely stunted due to excess phosphorus. Infected plants showed essentially the same growth response to the phosphorus treatments as healthy plants. Initial symptoms appeared at the same time in each treatment and no differences in severity of symptoms among treatments could be distinguished.

In 1 experiment in which untreated sap was tested by lesion assays there was in general an increase in virus concentration with an increase in phosphorus. The lowest virus concentration occurred at the lowest phosphorus level and the highest concentration usually at the highest phosphorus level but the virus concentration at 547 p.p.m. was not significantly greater than at 237 p.p.m. Differences between all other treatments were significant.

When virus concentration was determined by ultraviolet absorption of purified preparations and by lesion assays of the inocula after clarification and also after purification there was a direct correlation between virus concentration and additions of phosphorus. In all assays (Table 4) the maximum virus concentration of the expressed juice and the purified fraction occurred at the highest phosphorus level and the lowest concentration at the lowest level. Analysis of variance revealed no significant differences in lesion counts at 237 and 547 p.p.m. Since optical density readings did vary considerably between these 2 levels, it might be reasoned that some of the virus at the high phosphorus levels was noninfective or that some inhibitive substance was produced at phosphorus levels above 237 p.p.m.

Potassium.—Only 1 experiment was conducted with potassium and in it plants were grown at 8, 78, 704 and 1408 p.p.m. Plants were harvested 25 days after inoculation and their response, in fresh weight, to the various nutrient levels was recorded at that time. The least growth was recorded at 8 p.pm. and the maximum at 704 p.p.m. Growth was reduced at 1408 p.p.m. The actual differences in growth among the various levels, however, were not great.

Lesion assays were made of crude extracts of systemically-infected tip leaves, of heat-clarified extracts of whole plants, and of purified virus preparations from whole plants. In addition the optical density of each purified fraction was determined.

There were only slight differences (Table 5) in virus activity between any of the potassium levels. However, lowest lesion counts were consistently found to be at the highest and lowest potassium levels where the least growth occurred and the highest counts at 78 and 704 p.p.m. levels where maximum growth was recorded. These differences, however, were not sig-

TABLE 4.—*The effect of phosphorus nutrition on the concentration of tobacco mosaic virus in tobacco*

Phosphorus levels (p.p.m.) and assay intervals	Average fresh weight per plant (gm.)	Virus activity of clarified juice [a]	Virus activity of purified fractions [a]	Optical density of purified fractions [b]
24 days [c]				
3	6.4	88.2	63.2	0.406
93	13.3	149.3	98.9	0.932
237	15.9	147.9	111.5	0.950
547	8.4	163.4	115.0	1.110
36 days				
3	7.7	114.3	89.9	0.219
93	16.8	221.9	135.3	0.870
237	18.3	243.8	149.2	0.910
547	10.7	281.4	176.4	1.200
43 days				
3	9.6	101.3	70.8	0.520
93	18.2	193.6	141.4	0.915
237	20.8	220.1	152.3	1.015
547	11.1	216.4	193.6	——[d]

[a] Virus activity refers to infectivity as measured by the local lesion reaction. Values shown are averages of 9 half-leaf replications.
[b] Ultraviolet wave length of 265 mμ.
[c] Days after inoculation.
[d] Beyond the limit of the spectrophotometer scale.

nificant. Optical density readings likewise showed higher concentrations of virus at the 78 and 704 p.p.m. levels and lower concentrations at 8 and 1408 p.p.m. However, the actual differences are considered too slight to be significant. The response in host growth and virus concentration to potassium in this single experiment was less marked than in previous studies (2, 5).

Concentration of balanced solutions. — Tobacco plants were grown in a balanced solution of 4 concentrations (0.5H, 1H, 2H and 3H) as described in previous papers (2, 5). The 1H was the basic solution and represented N, P, K levels of 210, 31, and 235 p.p.m., respectively. The concentration of trace elements and the pH (6.0—7.0) were constant in all solutions.

In several experiments the maximum growth response of both infected and noninfected plants occurred at the 1H level and the least growth occurred at the 3H level. Growth at the 0.5H and 2H levels was similar. In 1 experiment, however, plants at 0.5H at 26 days after inoculation weighed more than plants at the 2H level whereas at 30 and 42 days after inoculation the reverse was true (Table 6). Plants at 2H and 3H were much darker green than normal. Plants at 3H were constantly wilted, due to the high osmotic value of the nutrient solution. During the hot portion of the day the plants at 2H were also wilted, but recovered when temperatures became lower.

Mosaic symptoms appeared at approximately the same time in the various treatments. However, there was a gradient in ultimate symptom expression ranging from the mildest symptoms at 3H to the most severe at 1H. Symptoms at 2H and 0.5H were slightly less severe than at 1H.

In 1 experiment in which the virus multiplication in systemically-infected tip leaves was tested by lesion assays, there was a direct correlation between vegetative growth and virus concentration. The highest virus concentration in all assays occurred at the 1H level where growth was the greatest. The virus concentrations in plants grown at 0.5H and 2H did not differ significantly. The fewest lesions were obtained from plants grown at 3H where the least growth was recorded. Virus concentration curves were similar in all assays and likewise the virus concentration, host growth, and symptom severity were correlated in all

TABLE 5.—*The effect of potassium nutrition on the concentration of tobacco mosaic virus in tobacco*

Potassium levels (p.p.m.)	Average fresh weight per plant (gm.)	Average number of lesions[a] per half leaf with inocula taken from—			Optical density of purified fractions [d]
		Young leaves [b]	Whole plants [c]	Purified fractions	
8	8.5	232.4	169.5	190.9	0.640
78	10.3	259.2	173.7	221.3	0.670
704	11.2	243.1	192.5	209.7	0.655
1408	9.6	214.9	143.4	181.8	0.608

[a] Values shown are averages of 9 half-leaf replications.
[b] Assay made at 5 days after inoculation.
[c] Plants were harvested 25 days after inoculation.
[d] Ultraviolet wave length of 265 mμ.

TABLE 6.—*The effect of 4 concentrations of balanced solutions on the concentration of tobacco mosaic virus in tobacco*

Nutrient concentrations	Average fresh weight per plant (gm.)	Virus activity of clarified juice [a]	Virus activity of purified fractions [a]	Optical density of purified fractions [b]
26 days [c]				
0.5H [d]	12.8	122.8	77.1	0.344
1.0H	14.4	193.2	161.1	0.980
2.0H	11.3	114.1	81.2	0.820
3.0H	7.2	78.1	74.2	0.780
30 days				
0.5H	14.6	110.2	102.4	0.472
1.0H	19.8	184.1	168.7	0.682
2.0H	16.2	163.4	150.4	0.590
3.0H	8.1	70.9	92.7	0.403
42 days				
0.5H	16.4	133.4	113.4	0.203
1.0H	21.3	272.8	240.0	0.453
2.0H	19.9	197.8	182.3	0.410
3.0H	9.3	82.7	171.3 [e]	0.392

[a] Virus activity refers to infectivity as measured by the local lesion reaction. Values shown are averages of 9 half-leaf replications.
[b] Ultraviolet wave length of 265 mμ.
[c] Days after inoculation.
[d] H refers to Hoagland's solution.
[e] In analysis of treatment comparisons this value was significantly lower than 2H and not significantly different from 0.5H. See page 79 for an explanation of the failure of these figures to reflect differences accurately.

assays.

Other experiments were run in which the virus concentration of whole plants was assayed by both local lesion inoculations and ultraviolet absorption (Table 6). Results of the lesion assays of the purified virus suspensions and clarified juice showed that the virus concentration curve coincided with the growth curve and approximated the data obtained with untreated sap from systemically-infected tip leaves. In other words at 1H, where maximum growth occurred, the virus concentration was also highest and at 3H, where the least growth occurred, virus concentration was reduced.

The optical densities of the purified virus fractions were not correlated as closely with the vegetative growth as were lesion counts. With half-leaf inoculations with the untreated expressed juice any decrease in growth was accompanied by a decrease in the lesion counts and vice versa. The least growth always occurred at 3H and similarly the lowest virus concentration. The optical density of each virus suspension from plants at 3H, with the exception of the 30-day harvest, was about double that of the virus suspension from plants at 0.5H even though plants at 0.5H were considerably larger than plants at 3H. However, maximum optical density readings always were obtained from virus suspensions of plants at 1H followed by those from plants at 2H.

Since the ultraviolet absorption accounts for active, noninfective, and aggregated virus particles whereas the lesion assays measure only active virus, the high concentration of virus at the 3H level when measured by ultraviolet absorption would indicate that there was inactive virus at the 3H level which was not measured by lesion assays.

DISCUSSION.—The present study was made with the purpose of exploring the contradictory conclusions (1,3,6,7,8,9) regarding the effect of nitrogen nutrition on multiplication of tobacco mosaic virus in tobacco and to expand unconfirmed data on similar effects of phosphorus and potassium.

All assays of clarified sap and purified preparations showed the virus concentration to be directly related to the amount of nitrogen supplied the host, even though marked stunting occurred at the highest nitrogen level. Lesion assays from untreated expressed saps showed a reverse gradient. This discrepancy is explained by the presence of an inhibitor in plants which apparently increased with increase of nitrogen and thus increasingly inhibited the virus.

Spencer (6) also found that concentration of tobacco virus 1 in tobacco depended directly on the amount of nitrogen supplied to the host. His lesion counts indicated that virus concentration was greatly reduced in the expressed juice of nitrogen-deficient plants as compared to juice receiving ample nitrogen and that the highest concentration of virus was from plants receiving excess nitrogen even though plant growth was inhibited. He further stated (9) that interruption of the nitrogen supply to systemically-infected plants not only inhibited further virus multiplication but also inactivated the virus already present, thus leading to a reduction in infectivity. Chessin (3) also showed a reduced infectivity to tobacco virus 1 in nitrogen-deficient tobacco plants when infectivity was measured on Golden Cluster bean but not when *N. glutinosa* was used as the assay host.

Bawden and Kassanis (1) in their studies with tobacco mosaic virus found no evidence that there was a

change in infectivity of virus from plants receiving different amounts of nitrogen. They also reported that with tobacco plants grown in fertile soil, to which was added various nutrients, nutritional conditions most favorable to plant growth were those most favorable to virus multiplication. However, it is well to note that in their soil studies they did not use nutrient levels high enough to effect stunting. Additions of nitrogen to the soil did not increase virus concentration or plant growth unless phosphorus was also added. This would indicate that they were, perhaps, using a phosphorus-deficient soil which restricted the effect of the nitrogen. This is further suggested in their nutrient controlled sand culture experiments in which their data show that increases in nitrogen without increased phosphorus would affect virus concentration and that nitrogen levels high enough to cause stunting did not similarly retard virus activity but on the contrary increased it.

It was reported with turnip virus 1 in *N. glutinosa* and *N. multivalvis* (5) and with cucumber virus 1 in spinach (2) that when nitrogen nutrition was varied virus multiplication closely paralleled host growth; excess nitrogen decreased both virus concentration and plant growth. The decrease of virus at excess nitrogen levels in those cases was probably an indirect effect due to the harmful effect of the excess nitrogen on metabolic processes in the cell. The synthesis of tobacco mosaic virus apparently was not influenced by the harmful effect of the excess nitrogen on plant growth. Concentration assays in the studies with turnip virus 1 and cucumber virus 1 were, however, only by local lesion tests with crude extracts. In the present studies with tobacco virus 1, involving local lesion assays, it was also found that expressed crude saps from the highest nitrogen level were much less infectious than extracts from lower nitrogen levels. This was attributed to some type of inhibitor that is produced with excess nitrogen. It may well be that in the case of turnip virus 1 and cucumber virus 1 an identical situation occurred and that the local lesion assays did not indicate the total virus present.

The results with phosphorus in general agreed with the results of other workers. Increases in phosphorus were paralleled by corresponding increases in virus concentration. At the highest phosphorus level virus concentration was as high as or higher than at any other level even though plants at the highest phosphorus level were stunted. With turnip virus 1 in *N. glutinosa* and *N. multivalvis* (5) and with cucumber virus 1 in spinach (2), the highest concentration of virus occurred at the highest phosphorus level regardless of which level showed maximum growth. Bawden and Kassanis (1), in sand cultures, also showed higher virus concentrations at an excess phosphorus level where plant growth was inhibited.

Phosphorus, like nitrogen, is an essential part of the virus protein and as such it is supposed that phosphorus variation would affect virus activity in much the same manner as nitrogen. A deficiency of phosphorous should limit virus synthesis first by limiting the necessary building material and secondly by upsetting certain vital functions of the cell. However, virus production apparently was not hindered at the excess phosphorus levels even though plant growth was markedly affected. A much higher level of phosphorus and of nitrogen is required to retard virus multiplication than to inhibit plant growth. There was some indication, however, that at the highest phosphorus level infectivity was either reduced or some inhibitor was developed that reduced the activity in expressed sap.

With variation of potassium levels and with different concentrations of balanced solutions it was found that, as was also the case with turnip virus 1 (5) and cucumber virus 1 (2), the effect on virus concentration mainly reflected responses in host growth.

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LEAF PENETRATION BY PHYTOPHTHORA INFESTANS [1]

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SUMMARY

Histological studies of leaf penetration of susceptible Cobbler and resistant Kennebec potato and Wisconsin "55" tomato by potato race A of *Phytophthora infestans* showed that before penetration occurred, the zoospores encysted, germinated and produced appressoria.

Appressoria were slightly smaller than encysted zoospores. The protoplasm from an encysted zoospore flowed through the germ tube into the appressorium. The protoplasm was then cut off from the empty spore and germ tube by the wall of the spherical appressorium. The empty spores and germ tubes were usually sloughed leaving the appressoria. These appressoria could easily be mistaken for encysted zoospores and lead to the conclusion that penetration had taken place directly without the aid of appressoria.

A minute infection peg was produced on the underside of the appressorium which entered the epidermal cell and established a primary mycelium. Protoplasm passed from the appressorium through the infection peg to form the primary mycelium. Finger-like secondary mycelium developed which spread throughout the tissue in susceptible Cobbler leaves. Appressorium formation by encysted zoospores was also observed in Petri dishes containing a small amount of water.

Penetration was accomplished 2 hours after inoculation with motile zoospores on both resistant and susceptible hosts. In most cases penetration occurred through the epidermis (upper and lower) directly, but occasional penetration through stomata was observed.

Typical spreading-type lesions developed on susceptible Cobbler leaves, but pin-point necrotic spots developed on resistant Kennebec leaves. Points of penetration were more numerous on Cobbler than on Kennebec leaves. In sections of Kennebec leaves, the mycelium was never observed beyond the cell originally penetrated. Disintegration of the primary mycelium and death of cells surrounding the point of penetration occurred between 48 and 72 hours after inoculation.

Although indentation of cell wall by the infection peg was observed, indicating that mechanical pressure was responsible for entry, sections revealed that some substance was acting in advance of the invading hyphae. Cell walls below the point of penetration or below a clump of germinating zoospores showed an increase in affinity for the safranin stain.

Attempts to observe penetration by germ tubes from sporangia failed, even though long germ tubes were found growing on the surface of the leaves.

Extensive reviews of the literature pertaining to the life history of the late blight fungus have been published (4, 5, 8, 9, 10), but little work on leaf penetration is available. De Bary (5) was one of the first workers to describe zoospore penetration on potato stems. His observations indicated that zoospore penetration was direct and that it occurred about 2 hours after inoculation with zoospores. His drawings show a small enlargement at the end of the germ tube but no mention was made of appresorium formation. Ward (16) in 1887 published detailed sketches showing the zoospores penetrating the host directly through the epidermis and also by way of the stomata. Mechanical pressure by the germ tube from the encysted zoospore seemed to be the mechanism of penetration. The time required for penetration was not mentioned.

Barrus (1) reported that zoospores produced germ tubes and secreted a substance which dissolved the walls of the epidermis to gain entrance into the epidermal cell of potato leaves. The drawings of Barrus show a rather long germ tube penetrating the epidermal cell wall, but the length of time required for this process is not mentioned. Berg (2), working with tomato and potato strains of the fungus, observed that zoospore penetration and infection required about 3½–5½ hours.

Although Melhus (9) suggested that the number of stomata may influence the amount of infection, Crosier (4) found, after sectioning many inoculated leaves, that penetration occurred almost entirely through the epidermal cell walls. He also found that, in the case of the immune *Solanum demissum*, the swarmspore germ-tube had penetrated directly through the epidermal cell wall of the upper leaf surface. Röder (14), working with tomato, has shown that zoospores penetrate the upper surface of tomato leaves in 5 hours. Drawings by Röder show that penetration is accomplished by means of a minute infection peg. He also shows the primary mycelium spreading out in an irregular mass and penetrating to the depth of 1 epidermal cell in a 5-hour period. Intracellular branching and intercellular spreading of infection hyphae are also shown in the flesh of a tomato fruit. Müller (12) has shown that leaves of several non-Solanaceous plants may be penetrated but that further development of mycelium was restricted.

The purpose of this paper is to report the observation of appresorial formation by *Phytopathora infestans* (Mont.) de Bary as an aid in penetration and the reaction of resistant and susceptible potato leaves to infection.

METHODS AND MATERIALS.—The isolate of *P. infestans* used in these studies was obtained in 1949 from an infected tuber of the Katahdin variety. It was main-

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tained on lima bean agar (6) at 20° C. Cultures used for inoculation were 12–14 days old. The isolate was identified as the common potato field strain (race A) by inoculating Marglobe and Wisconsin "55" tomato plants and a set of differential potato hosts obtained from Mills and Peterson (11). Typical spreading lesions were formed on Cobbler leaves, while flecks and non-sporulating lesions were formed on the other differential potato hosts. Marglobe and Wisconsin "55" tomato leaves formed small non-sporulating lesions.

Potato leaflets used were obtained from field plantings of susceptible Cobbler and resistant Kennebec varieties. Leaves of Wisconsin "55" tomato were obtained from greenhouse-grown plants. The potato leaves were collected just before routine application of fungicides and thoroughly rinsed in distilled water before being used for inoculation. The leaflets were placed in moist Petri dishes and both sides were thoroughly sprayed with a suspension of viable zoospores.

The inoculum was prepared by flooding test tube cultures of the fungus with about 15 ml. of distilled water to obtain a heavy suspension of sporangia. This suspension was strained through fine cheesecloth and incubated in Petri dishes at 10° C. for about 2 hours until a large number of zoospores were liberated by the sporangia. Prior to spraying the leaves, the zoospore suspension was diluted with an equal volume of distilled water and transferred to a small hand-type deVilbis atomizer.

The leaf tissue was inoculated at room temperature and incubated in covered Petri dishes at 20° C. The inoculated tissue was removed at 2, 6, 12, 24, 48 and 72 hour periods. Incubation was carried out in total darkness except for brief periods of time consumed in removing the desired plates.

At the end of each period, leaves were removed from the Petri dishes and sprayed with a thin coating of pure egg albumin to prevent the zoospores from being washed from the surface of the leaflets during the dehydrating process. Sections treated with egg albumin showed that the coating was in close contact with the epidermis throughout the entire section. Immediately after the egg albumin treatment, the leaflets were placed in F.A.A. fixing solution. After fixation, the tissue was passed through a tertiary butyl alcohol dehydration schedule (15) which was modified slightly by using absolute alcohol in place of 95% alcohol.

The inoculated tissue was then embedded in paraffin, and cut on a microtome at thicknesses of 3–10μ. All sections were stained with Conant's quadruple stain containing safranin, fast green, gold orange and methyl violet. The staining schedule employed was that given by Johansen (7) with minor variations. Permanent mounts were made in Canada balsam.

The inoculated tissue used for free-hand sectioning was removed from the incubator and sectioned with a sharp double edge razor. Free-hand sections were made of Cobbler and Kennebec potato leaves and the Wisconsin "55" tomato leaf. Camera lucida drawings were made of these sections 2 and 6 hours after inoculation with zoospores. No free-hand sections were made of tissue incubated beyond 6 hours.

EXPERIMENTAL RESULTS.—*Observation of susceptible Cobbler potato leaf sections.*—Paraffin sections prepared from Cobbler leaves 2, 6, 12, 24, 48 and 72 hours after inoculation showed progressive development of mycelium within the tissue from the time of penetration through formation of typical spreading lesions.

Examination of 2- and 6-hour sections showed that germ tubes from encysted zoospores formed appressoria before penetrating the epidermal cell walls (Figs. 1A, 1B and 2A). The appressoria formed at the distal end of encysted zoospore germ tubes were closely affixed to the surface of the leaf. They were slightly smaller than the original zoospore. Some sections indicated that penetration was accomplished by mechanical pressure exerted by an infection peg. Figure 1C shows indentation of the cell wall at the beginning of penetration, and Figure 1D shows the infection peg as it enters the epidermal cell.

The sections prepared from leaves 2 hours after inoculation showed that a few of the zoospores had encysted, formed germ tubes and appressoria, and had established primary mycelium in the epidermal cells. Most of the spores, however, were in the process of germinating and were beginning to form appressoria (Fig. 1A).

The slides prepared from 12- and 24-hour inoculated leaves showed a larger number of penetration points than did sections made from 2-hour inoculated leaves. A few of the penetration points showed that finger-like projections of secondary mycelium had developed from the primary mycelium (Fig. 2C). The 12-hour sections revealed that germ tubes varied in length. Those from spores which had penetrated 2 hours after inoculation were shorter than those from spores which did not penetrate until 12 hours after inoculation.

The 48- and 72-hour sections showed large areas of dead host tissue. The mycelium had advanced freely through the mesophyll and palisade tissue. There was no evidence of encysted zoospores and appressoria on the surface of leaves 72 hours after inoculation.

Observations showed that the fungus usually penetrated the host tissue directly, but there were a few cases where stomata were entered (Fig. 2B).

During the process of germination and penetration, protoplasm moved from the encysted zoospore through the germ tube into the appressorium. In some stages the protoplasm extended back a short distance in the germ tube, and in others it was completely enclosed by the wall of the appressorium. In the former case the protoplasm was separated from the empty spore and germ tube by a membrane which, in a few cases, could be mistaken for a cross-wall in the germ tube. As the infection peg entered the epidermal cell, protoplasm began to move inward and formed the primary mycelium which immediately began to enlarge and form secondary mycelium (Fig. 2C). The original

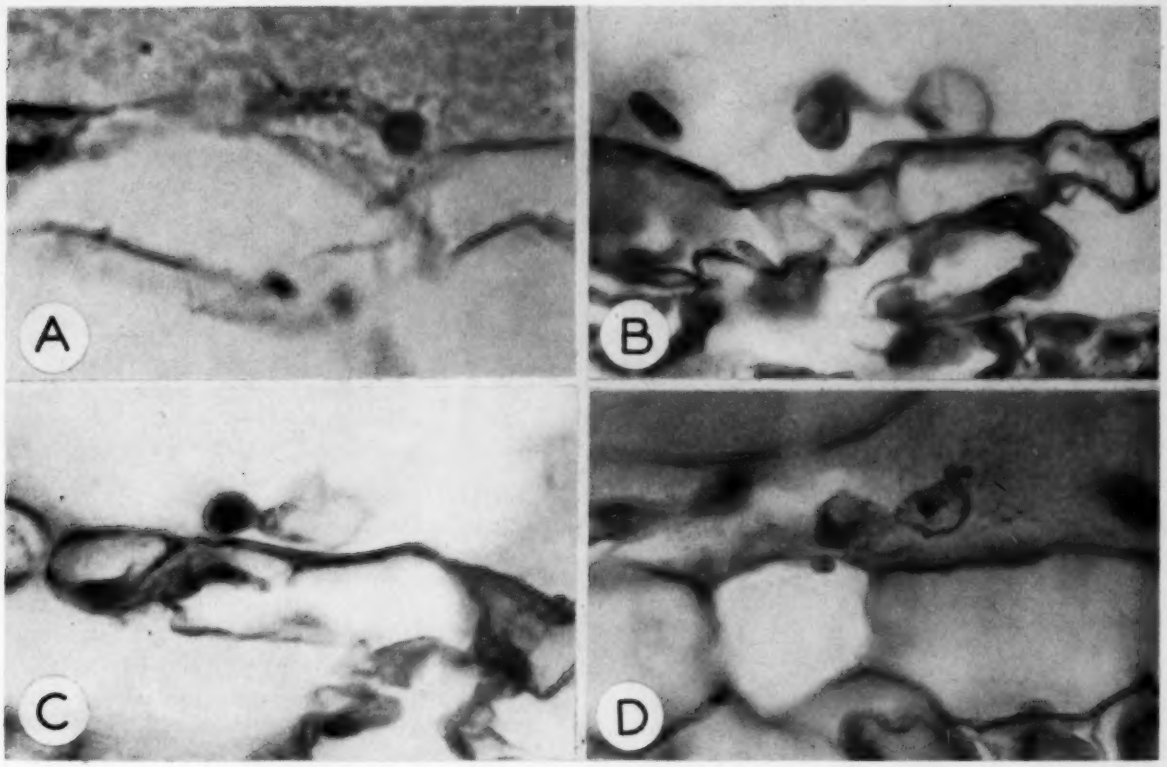

FIG. 1. Photomicrographs of stained sections of Cobbler potato leaves showing steps in penetration by *P. infestans* zoospores. A) Encysted zoospore showing germ tube and immature appressorium; B) germ tube and immature appressorium pulled away from the epidermal cell wall; C) beginning of penetration showing indentation of epidermal cell wall by infection peg produced by appressorium; D) the infection peg entering the epidermal cell.

encysted zoospore and germ tube were colorless and were sloughed easily after protoplasm had moved into the epidermal cell.

Near the point of penetration, the cell walls showed an increased affinity for the safranin stain. They also showed an affinity for safranin stain where a group of germinating spores rested on the epidermal surface (Fig. 3A). This indicates that some change may take place in cell walls in advance of penetration.

In 2 attempts to observe penetration by direct germination of sporangia, Cobbler potato leaves were inoculated with a sporangial suspension and incubated at 25° C. Free-hand sections were made 12, 24 and 48 hours after inoculation. Penetration by direct germination was not seen even though the long germ tubes were abundant on the leaf surface. Some of them formed secondary sporangia. Penetration was seen but in all cases was accomplished by the few zoospores liberated at 25° C.

Observation of resistant Kenebec potato leaf sections.—The Kennebec potato leaves, resistant to the isolate used, form only small necrotic flecks as a normal symptom (Fig. 3B). Examination of stained sections of Kennebec leaves revealed that the fungus penetrated the tissue in the same manner as it did Cobbler leaves. A primary mycelium was formed in the epidermal cell, but advance of secondary mycelium from the epidermal cell into other cells was not observed. Necrosis of cells immediately below the point of penetration occurred, however.

As was the case with Cobbler leaves, some spores had formed appressoria and penetrated the epidermal cell wall of Kennebec leaves 2 hours after inoculation. The 24- and 48-hour sections showed that necrosis of cells below the penetrated epidermal cell occurred, but that the fungus mycelium had not advanced. Seventy-two hours after inoculation, mycelium could not be found in the necrotic tissue nor in the surrounding healthy tissue, indicating that disintegration occurred while still in the epidermal cells.

Observation of tomato leaf sections.—Free-hand sections of Wisconsin "55" tomato leaves showed that zoospores encysted, germinated, formed appressoria, and penetrated in the manner described for Cobbler potato leaves (Fig. 2A). No attempts were made to observe further invasion of the tomato leaf tissue in the free-hand sections.

Appressoria formation in Petri dishes.—While preparing suspensions of zoospores for inoculation, it was noted that some encysted zoospores formed appressoria in Petri dishes. The protoplasm in the appressorium was separated from the empty spore and germ tube by a membrane. The length of the germ tubes varied considerably.

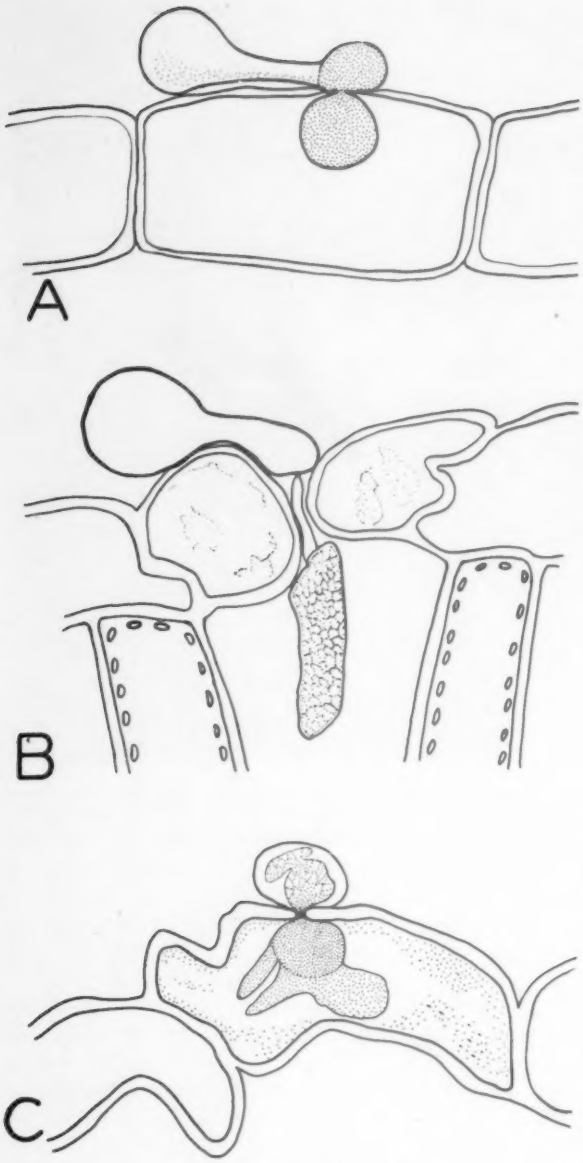

FIG. 2. Camera lucida drawings of penetration of Cobbler potato leaves by *P. infestans* zoospores: A) penetration of epidermal cell wall showing appressorial formation and formation of primary mycelium; B) penetration through stomata; C) finger-like projection of secondary mycelium formed from the primary mycelium. The original encysted zoospore and germ tube were lost.

DISCUSSION.—In this histological study, it was found that the mode of penetration by *P. infestans* is different from that previously described. The papers of de Bary (5), Barrus (1), Ward (16) and Röder (14) showed encysted zoospores germinating, producing a germ tube and entering the host by the extended germ tube. There was no mention of an appressorium or any similar organ by the germinating spore. De Bary showed an enlargement at the end of a germ tube which may have been an appressorium, although it was not recognized as such.

It was distinctly shown by this study that the germinating zoospore forms an appressorium before penetration. It is quite possible that the early workers saw appressoria, but mistook them for encysted zoospores. After the fungus forms an appressorium, the germ tube and the zoospore are void of protoplasm and may be sloughed, leaving only the appressorium, which is slightly smaller but similar in shape and in stain absorbed to the original zoospore. Since the appressorium and encysted zoospore are similar, it is easy to see how some of the early observations could have been misleading. Once all the protoplasm is located in the appressorium, it is difficult to see the outline of the original zoospore and the germ tube.

The time required for penetration is also noteworthy. De Bary (5) found that penetration occurred 2 hours after zoospores were placed on potato leaves, while Röder (14) reported tomato leaf penetration 5 hours after inoculation. The work herein shows that penetration occurs as early as 2 hours after inoculation on leaves of both resistant and susceptible potato varieties and on tomato leaves and confirms the time reported by de Bary. It may occur in less than 2 hours, since no sections were made within 2 hours of inoculation.

It is also worth noting that the method of penetration for resistant and susceptible varieties of potatoes is the same.

Appressoria formation by other members of the Peronosporales has been recorded. Yarwood (17) showed that germ tubes from sporangia of *Peronospora destructor* formed appressoria before penetrating stomata of onion leaves. Chu (3) observed that germ tubes from sporangia of *Peronospora brassicae* formed appressoria before penetrating the epidermis through the boundary between 2 cells of crucifer leaves.

Evidence shown herein (Fig. 1C) indicates that mechanical pressure by the infection peg protruding from the appressorium may be responsible for actual penetration. However, it was observed that cell walls near the point of penetration showed an increased affinity for safranin stain. In addition, it was noted that where a clump of encysted zoospores were germinating on the surface of the leaf, the cell walls of the epidermis and palisade parenchyma immediately below showed an increased affinity for safranin stain (Fig. 3A). This indicated that some substance may be released during the process of germination which causes a change in composition of cell walls in advance of the invading infection peg and is in line with the observations of Barrus (1). It is possible that mechanical pressure is responsible for penetration of the cuticle and that enzymatic action alone or in combination with mechanical pressure is responsible for penetration of the remainder of the epidermal cell wall.

In Kennebec leaves, the fungus mycelium was never observed in cells other than the epidermal cells originally penetrated. Seventy-two hours after inoculation, the primary mycelium could not be detected in the epidermal cells of Kennebec leaves, while comparable

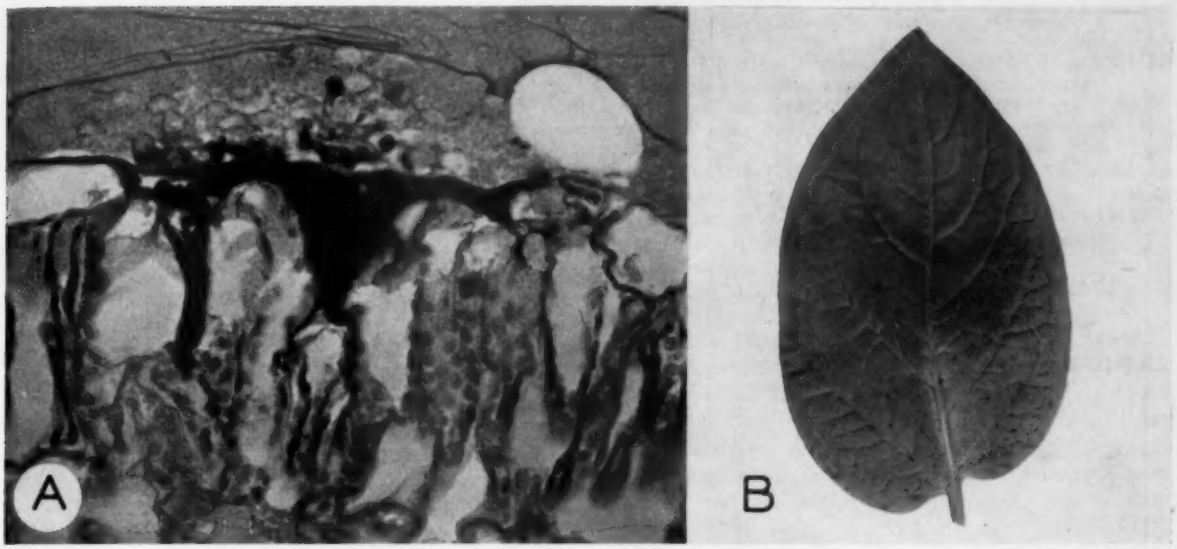

FIG. 3. A) Necrosis of Cobbler potato leaf cells below a group of germinating zoospores; penetration had not occurred. B) Necrotic-fleck reaction of Kennebec potato leaflet when inoculated with zoospores of *P. infestans* potato race A.

sections of Cobbler leaves showed abundant mycelium advancing through the tissue. Apparently, disintegration of the primary mycelium occurred while it was still in the epidermal cell of Kennebec leaves.

It was not possible to determine definitely the mechanism of resistance exhibited by Kennebec leaves in these studies. Since it is evident that some substance is secreted by germinating spores (Fig. 3A), it is possible that the same or a different substance may initiate a resistant reaction in leaf cells of Kennebec and other resistant potato varieties. Perhaps the resistant reaction is similar to that reported by Müller (12) and Müller and Behr (13), who observed cell collapse and infiltration of cell walls and cytoplasm with phlobaphene-like compounds which were toxic to the invading hyphae. The chief difference found by Müller and Behr between resistant and susceptible varieties was in the rate of reaction; the resistant varieties produced the toxic compound in advance, while the susceptible varieties produced the toxic substance behind the invading hyphae. According to these authors, the cells of resistant varieties showed a hypersensitive-type reaction to infection. On the other hand it is possible that the resistance may be a matter of nutrition. It is obvious that the mycelium formed in a resistant host soon disintegrates and disappears. If the mycelium is unable to obtain suitable food from a resistant host it would die of starvation, and the autolytic products would be sufficient to account for the necrosis of the penetrated cell and those adjacent to it.

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A PHYTOPATHOLOGICAL NOTE

Seed Transmission of Tobacco-Ringspot Virus in Lincoln Variety of Soybean. P. R. DESJARDINS, R. L. LATTERELL, AND J. E. MITCHELL. The limited and erratic spread of bud blight disease, caused by tobacco-ringspot virus, in Lincoln variety of soybean in the field suggested that something other than insect transmission was involved. This virus has been demonstrated to be transmitted through seeds of tobacco and petunia.[1,2] Greenhouse tests were undertaken to determine whether tobacco-ringspot virus is transmissible through seeds of soybean.

Seeds produced in the greenhouse on Lincoln soybean plants infected with tobacco-ringspot virus were collected over a period of time and planted in 2 lots. Each lot was of necessity small since under greenhouse conditions Lincoln soybeans severely infected with the virus produced few, if any, seeds.

The first lot of 50 seeds was planted in the greenhouse during the fall of 1951. Thirty-seven of the 42 plants which emerged had an atypical appearance in the seedling stage as illustrated by the plant on the right in Fig. 1. Four of these 37 died before the first trifoliolate leaves had formed. All the remaining 33 plants developed symptoms typical of bud-blight as described by Allington.[3] An assay for the virus, using cowpeas and soybeans, revealed the presence of tobacco-ringspot virus in all plants showing symptoms.

In the second planting (25 seeds), made in late spring of 1952, only 9 of 24 plants which emerged were abnormal in appearance and only 3 developed typical symptoms. Due to the small number of plants in the latter group, all 24 plants were assayed on cowpeas and 13 of the 24, including the 3 that showed symptoms, were found to contain the virus.

[1] Valleau, W. D. 1932. Seed transmission and sterility studies of two strains of tobacco-ringspot. Kentucky Agr. Exp. Sta. Bul. 327.

[2] Henderson, R. G. 1931. Transmission of tobacco-ringspot by seed of petunia. Phytopathology 21: 225-229.

[3] Allington, W. B. 1946. Bud blight of soybean caused by the tobacco-ringspot virus. Phytopathology 36: 319-322.

FIG. 1. Seedlings of Lincoln variety soybean of the same age. LEFT: grown from seeds obtained from a healthy plant. RIGHT: grown from seeds obtained from an infected plant and showing the symptoms of the seed-transmitted virus.

The results indicate that the virus was transmitted in 78 and 54 per cent of the first and second plantings, respectively. The fact that the seeds were obtained from obviously infected plants would explain the very high percentage of seeds apparently transmitting the virus. The difference between the 2 plantings was apparently due to a suppression of symptoms occasioned by the higher temperatures existing at the time of the second test. The 3 plants which developed typical symptoms were among a group which had been placed in a lower temperature house.

No instances of accidental infection of soybean plants by ringspot virus have been observed by the authors in the greenhouse, nor have such symptoms been apparent among the large number of plants grown during the period of these tests from seed of this variety obtained from commercial sources. Therefore, the results given above strongly indicate that seed transmission of this virus does occur in Lincoln variety soybean.—Department of the Army, Chemical Corps, Washington 25, D. C.

THE ASTER RINGSPOT VIROSIS OF CENTRAL FLORIDA [1]

C. W. Anderson

SUMMARY

Aster ringspot virus was found to have an extensive host range, and a thermal inactivation point of between 75 and 80°C./10 minutes. It causes ringspot symptoms in tobacco, local lesions only in cucumber, cucumber mosaic virus-type lesions in cowpea, and local and systemic symptoms in Sill and Walker's cowpea strain. The virus simulates cucumber mosaic virus in some respects but differs from it in producing no systemic reaction in cucumber plants, in failure of aphid transmission, and in displaying no immunological relationship with cucumber mosaic virus.

The following plants were found infected with the aster ringspot virus in the field: aster, pepper, pansy, and *Solanum gracile*. No previous report of the natural infection of *Allamanda cathartica* or of *Gloriosa superba* by cucumber mosaic virus has been noted, but a report of the experimental infection of *G. rothschildiana* was found.

Although aster ringspot virus is common in pepper in 1 area, it is not an important factor in pepper production. The fruit rings seen in peppers in certain areas probably result from infection by cucumber mosaic virus strains allied to *M. cucumeris commelinae* or to the pepper ringspot strain of Doolittle and Zaumeyer.

A disease of pepper (Capsicum frutescens L.) characterized by striking chlorotic ring, line, and oakleaf patterns was noted in many fields in the Sanford-Samsula area of central Florida in the spring of 1950. Affected plants were not noticeably stunted and bore normal fruit. The disease recurred in 1951 and 1952. Early in 1951, Azure blue aster plants (*Callistephus chinensis* Nees), affected by a similar disease, (Fig. 1) were received from Tampa. These plants were not noticeably stunted and the flowers were normal. The disease recurred in 1952. The incitant proved to be a sap-transmissible virus, and symptoms induced by both the aster and the pepper isolates in experimentally infected California Wonder pepper plants were identical. A single naturally infected aster plant served as the original source of inoculum for the work reported herein.

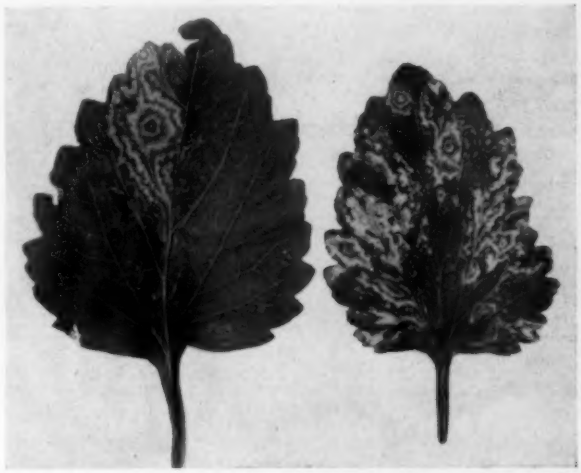

FIG. 1. Leaves of china aster plant naturally infected with ARSV, displaying brilliant yellow ring and line patterns. (Photograph by H. N. Miller)

MATERIALS AND METHODS.—Viruses used in these studies were: the aster ringspot virus (ARSV) from aster described herein; potato X virus (*Annulus dubius* (Holmes)) obtained from potato; cucumber mosaic virus isolate V1 (2, 3); an isolate of cucumber mosaic virus closely resembling the southern celery mosaic strain (*Marmor cucumeris commelinae* H.); the indicator strain of cucumber mosaic virus (*M. cucumeris judicis* H.); and tobacco ringspot virus (*Annulus tabaci* H.).

The strain of cowpea of Sill and Walker (15) was supplied by J. C. Walker. J. H. Freitag supplied seed of *Nicotiana glutinosa* L. H. N. Miller accomplished the single return inoculation to aster using healthy commercially grown aster seedlings as test plants and a nasturtium plant experimentally infected by the writer as inoculum source.

Young, vigorously growing plants were inoculated in most cases. Plants were sprayed with parathion and fertilized weekly. Nicotine sprays were used during studies of possible aphid transmission. One control plant was used for each test plant except during studies of the thermal inactivation point. Experimentally infected *Nicotiana tabacum* L. or *N. glutinosa* L. plants were used as virus sources in most cases. Attempts to recover the virus from the test species were made to one or the other *Nicotiana* species.

RESULTS.—*Host and symptoms.*—Mechanically inoculated plants were kept for at least 20 days. Most Marketer cucumber, all watermelon, and some celery plants were kept for at least 30 days before recovery was attempted. Attempts to recover virus were made from inoculated or noninoculated plant parts as desired. Two *N. tabacum* or *N. glutinosa* plants were inoculated in each recovery attempt. Half the control plants were inoculated with sap from control plants of the variety involved. Results of the host range inoculations are in Table 1.

Several factors influenced the results obtained, as

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The writer is indebted to Drs. H. N. Miller and G. Swank, Jr. for assisting with part of the work, to Dr. S. P. Doolittle for supplying a culture of the tobacco ringspot virus, and to Dr. R. H. Larson for identifying the potato X virus.

Florida Agricultural Experiment Station Journal Series, No. 181.

TABLE 1.—*Results of studies on host range of the aster ringspot virus*

Species inoculated	Results[a] of			
	Inoculations		Recovery attempts from	
	Plants infected	Local symptoms only	Inoculated parts	Noninoculated parts
Nicotiana tabacum L. var. White Burley	11/11	6	----	4/10
N. glutinosa L.	10/10	5	2/2	0/2
Lycopersicon esculentum Mill. var. Marglobe	12/27	0	----	10/14
Capsicum frutescens L.				
var. California Wonder	26/27	3	----	6/6
var. Tabasco	11/11	0	----	3/4
Cucumis sativus L.				
var. Marketer	20/20	20	7/10	0/12
var. A & C	8/8	8	4/4	0/8
Cucurbita pepo melopepo Alef. var. Early Prolific Straightneck	4/4	4	----	----
Citrullus vulgaris Schrad var. Cannonball	10/17	10	2/10	0/9
C. vulgaris citroides Bailey var. Colorado Preserving	5/8	5	0/2	0/2
Tropaeolum majus L. var. Golden Gleam	15/15	0	----	6/6
Zinnia elegans Jacq. var. Lilliput Golden Gem	12/35	0	----	4/12
Callistephus chinensis Nees	2/4	0	----	----
Apium graveolens L.	8/10	8	4/4	0/6
Vigna sinensis (L.) Endl.				
var. Blackeye	25/29	25	0/10	0/10
var. Black (Sill & Walker strain)	12/12	4	----	4/6
Phaseolus vulgaris L. var. Bountiful	10/23	10	0/8	0/8

[a] Results are tabulated as plants infected/plants inoculated. Determinations of the numbers of plants infected were made on the basis of symptoms induced, regardless of whether symptoms were local or systemic.

indicated by the following observations: 1) tomato plants inoculated during February and March developed only very mild symptoms and were essentially symptomless carriers of the disease, although the virus was readily recovered from systemically-invaded parts. Plants inoculated in early April developed a severe disease consisting of necrotic and chlorotic ring, line, and oakleaf patterns, chlorosis, and stunting. Those inoculated from late April to June developed no symptoms and no virus could be recovered from them. 2) The type of symptoms displayed by plants of *N. tabacum* and *N. glutinosa* varied according to the season; older plants of these species proved resistant to systemic invasion, especially when inoculated during the summer. 3) Infected plants of the *Nicotiana* spp., pepper, and zinnia often recovered from the symptoms of ARSV during the summer, and no virus proved recoverable from such plants. Therefore, most of the inoculations reported here were made between December 15 and April 15.

Plants of *N. tabacum* and *N. glutinosa* developed necrotic rings on inoculated leaves in 2–6 days and similar rings on systemically invaded leaves (Fig. 2). Chlorotic and necrotic areas often spread out from these rings. Necrosis was particularly evident along the veins. Tobacco leaves inoculated with apparently concentrated inoculum became almost entirely chlorotic, so that the number of necrotic lesions was impossible to count. Seriously affected, systemically invaded leaves became crinkled and distorted as a result of necrotic areas formed during leaf expansion; such plants if young were decidedly stunted. The color of the lesions varied, probably as a result of environmental factors.

Plants of 2 pepper varieties, china aster, and zinnia developed systemic chlorotic ring, line, oakleaf, and calico patterns. Lesions (chlorotic, sometimes concentric, rings in pepper and aster; chlorotic spots on young zinnia leaves) sometimes developed on inoculated leaves of all 3 hosts; systemic necrotic patterns occasionally developed in pepper and zinnia. Chlorotic patterns in pepper and aster were brilliant, often consisting of concentric rings. Chlorotic patterns in zinnia were mild, involved less of the area of affected leaves, and were composed of broader chlorotic lines. Single rings were more commonly noted. Zinnia leaves tended to outgrow their symptoms as they matured, but those of pepper and aster did not.

Nasturtium plants developed mild chlorotic patterns consisting of rings or spots or of chlorotic areas bor-

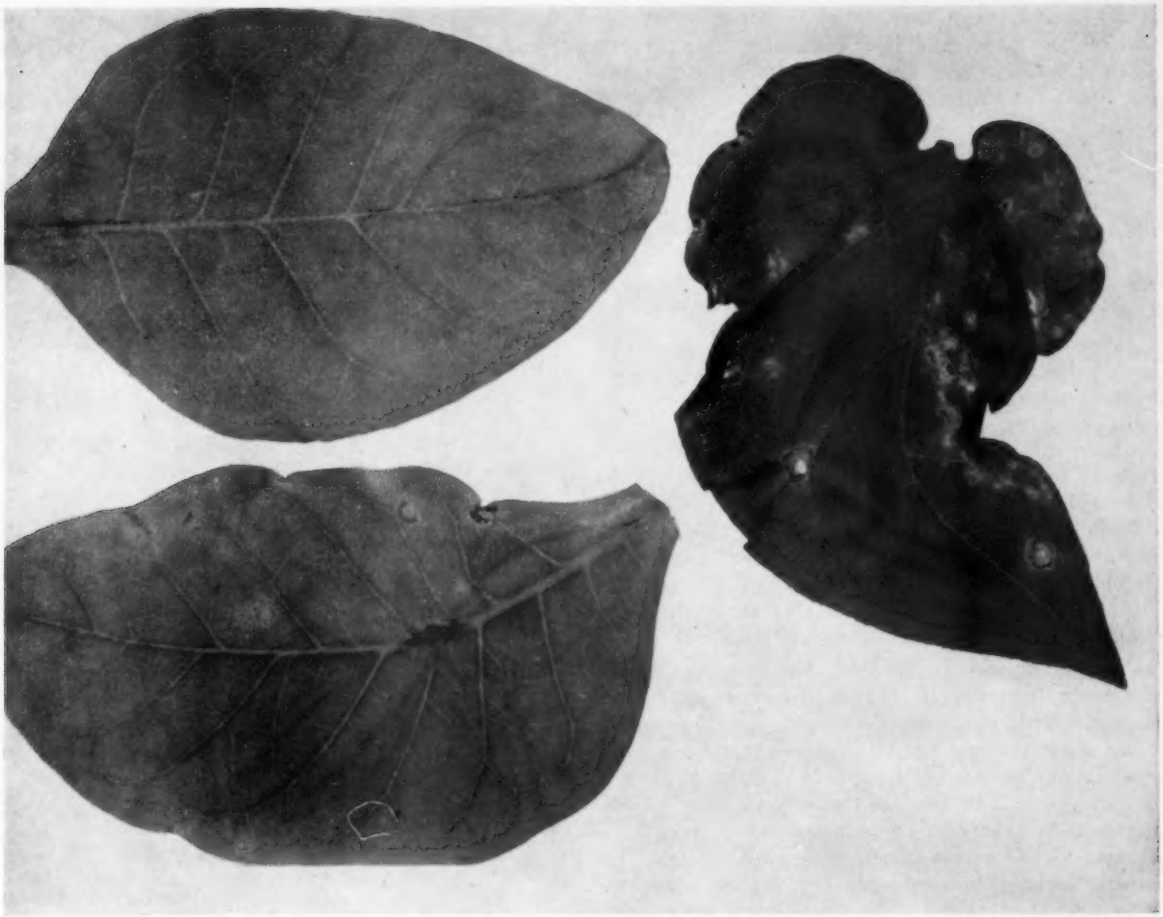

FIG. 2. Symptoms induced by the aster ringspot isolate. Lower left, ringspot symptoms in systemically invaded senescent tobacco leaf. Upper left, senescent healthy tobacco leaf. Right, ringspot symptoms in systemically invaded *N. glutinosa* leaf. Folding and tearing resulted from attempt to flatten out leaf distorted and crinkled by ARSV.

dering the veins, crinkling, stunting, and some necrosis.

Plants of 2 cucumber varieties, squash, watermelon, citron, celery, and bean developed symptoms only on inoculated cotyledons or leaves. Plants of cucumber, watermelon, and citron displayed large chlorotic rings or spots which tended to become necrotic. Cucumber cotyledons were often severely affected and sometimes died, and the resulting plants were stunted. No other symptoms were noted in noninoculated parts and the virus could not be recovered from them. Probably, the virus interfered so severely with metabolism and/or translocation in the cotyledons that the plants were stunted from lack of adequate nourishment. Squash developed smaller, less-defined spots or rings. Celery leaves, particularly the younger inoculated ones, displayed chlorotic spots, rings, or areas, and crinkling. Some necrosis was also noted. Bean leaves developed very small light brown necrotic spots in 2 days. Local symptoms in cucumber, watermelon, and citron, particularly, resembled those incited by the V1 isolate of cucumber mosaic virus.

Blackeye cowpea developed small red necrotic spots typical of cucumber mosaic virus infection. Sill and Walker's cowpea strain developed dull, irregular, poorly-defined chlorotic or red necrotic lesions on inoculated leaves and a brilliant yellow systemic mottle in which ring patterns were suggested. Inoculated leaves sometimes became generally chlorotic and crinkled.

Properties.—Two cc. samples of infective tobacco sap in thin-walled cotton-stoppered test tubes were heated in a water bath for 10 minutes at 70, 75, 80, and 85°C. and immediately cooled in tap water. Lesions were counted 10 days after inoculation. The average lesion counts of 4 separate experiments for unheated sap and for sap heated at 70, 75, and 80°C. were 437, 15, 3.8, and 0, respectively. Accurate counts were impossible on the plants inoculated with unheated sap, but the figures given are conservative. The thermal inactivation point lies between 75 and 80°C.

Typical lesions were induced in tobacco by infective *N. glutinosa* sap which had been frozen (0°F.) for 285 days. Longer intervals were not tried.

Aphid transmission.—Aphids from hibiscus, macroscopically identified as *Aphis gossypii* Glover, and aphids from turnip similarly identified as *Myzus per-*

sicae (Sulzer), and capable of breeding on pepper and tomato, were used in transmission tests. Forty-seven *A. gossypii* and 459 *M. persicae* failed to transmit ARSV from aster, pepper or tomato to pepper, tobacco, *N. glutinosa*, or cucumber plants. Short (seconds or minutes) acquisition feeding periods with preliminary fasting periods were used in some tests and long feeding periods were used in others. Most of the aphids could no longer be found on the test plants 24–48 hours after transfer, but a few remained for at least 4 days. ARSV did not prove aphid-transmissible under the test conditions.

Immunological studies.—Twenty or more days after inoculation, tobacco leaves systemically invaded by tobacco ringspot virus, potato virus X, or the V1 or indicator strains of cucumber mosaic virus were inoculated with ARSV. None of the viruses consistently reduced the number of lesions caused by ARSV. The cucumber mosaic virus isolates reduced the number of ARSV lesions by 81 per cent in 1 test, but in some others the plants infected with cucumber mosaic virus developed more lesions than the controls. In a reciprocal test, ARSV did not protect zinnia from the indicator strain of cucumber mosaic virus.

Natural hosts.—At Sanford, Samsula, or Tampa, *Capsicum frutescens* L., *Viola tricolor* L., and *Solanum gracile* Link displayed chlorotic symptoms resembling those induced by ARSV in certain hosts. Sap inoculations were made from field samples of each of the species to 2 or more of the following test plants: tobacco, *N. glutinosa*, pepper, and the 2 cowpea varieties. ARSV was obtained from all 3 species. The pepper isolates infected only pepper, but symptoms were typical of ARSV. The aster isolate proved difficult to transmit from pepper to other plants. It caused very few lesions in return inoculations to tobacco.

Allamanda cathartica L. from Sanford displayed chlorotic ring and oakleaf patterns of a somewhat different type and *Gloriosa superba* L. from Gotha showed chlorotic and necrotic leaf areas, stem streak, stunt, distortion, and broken flowers which displayed relatively small light-colored rings. Relatively typical strains of cucumber mosaic virus were secured from both species. The identity of both isolates was confirmed by transmission to cucumber and by immunological studies in zinnia.

Commelina nudiflora L. and *Ambrosia artemisiifolia* L. from Tampa and Sanford-Samsula, respectively, also displayed symptoms somewhat resembling those of ARSV. In 1953, *A. artemisiifolia* in Alachua County displayed similar symptoms and *Pelargonium hortorum* Bailey in the same county displayed symptoms strikingly suggestive of those of ARSV in pepper and aster plants. Sap inoculations did not disclose the presence of any virus in *Commelina*, *Ambrosia*, or *Pelargonium*. Infection of pepper (*Capsicum frutescens*) by ARSV in Alachua County was not discovered, although pepper is commonly grown there.

Field observations.—The presence of ARSV correlated with the presence of *Commelina* spp. and of neighboring aphid populations both in the Sanford-Samsula area and at Tampa. Wellman (25) listed *Commelina nudiflora* and *A. artemisiifolia* as sources of southern celery mosaic virus, and cucumber mosaic virus is more common in the Sanford area than elsewhere in central Florida. Thus, the presence of ARSV also correlated with the prevalence of cucumber mosaic virus.

ARSV spreads extensively in the winter, even among young aster and seedbed pepper plants. Observations indicate that infection of young pepper plants, and probably of aster also, results from field spread rather than from seed transmission. Young diseased pepper and aster plants commonly showed no evidence of damage by chewing insects. Thrips were not noted on seedbed pepper plants. Leafhoppers were occasionally present on young aster or pepper plants. Aphids were numerous on hibiscus at Tampa and on crucifers and other plants at Sanford and Samsula. They were found breeding on seedbed pepper plants. An extremely common or efficient vector is indicated, and aphids appear the only insects common enough to account for the early spread of ARSV.

Some pepper plants in the Sanford-Samsula area displayed yellow rings on the fruits. The leaves displayed symptoms resembling those seen in pepper plants infected by the V1 isolate of cucumber mosaic virus. The V1 isolate induced fruit rings in many pepper varieties in previously unreported tests, but ARSV never caused fruit rings in the greenhouse. Doolittle and Zaumeyer (7) reported a strain of cucumber mosaic virus which also caused fruit rings in pepper. ARSV cannot be considered an important factor in pepper production even though it is common in the Sanford-Samsula area. However, the virus decreased the salability of china aster plants in 1 planting. A possibility exists that the aster grower obtained ARSV in *Commelina* rhizomes included with compost material. Moreover, the wide host range of ARSV and its sometimes long incubation period in infected plants increase the likelihood that this virus may be shipped to new areas in various ornamentals including aster and perhaps *Pelargonium*, with possibly disastrous results for undesignated crops.

Discussion.—Over 20 viruses (5, 6, 9, 10, 11, 12, 13, 14, 16, 17, 18, 19, 20, 21, 23), including those of tomato bushy stunt, celery calico, spotted wilt, several viruses of the Annulaceae, and strains of several viruses of the Marmoraceae listed by Holmes (10), induce ringspot symptoms in tobacco. Some have been suspected of being strains of cucumber mosaic virus or of tobacco ringspot virus, whereas others are considered distinct. Most are separable from ARSV by their reactions on cucumber, their thermal inactivation points, or by the symptoms they induce in tobacco. Tomato bushy stunt virus differs strikingly from ARSV in the type of lesions induced in cowpea and probably also in the symptoms induced on tobacco. The lily ringspot (19) and Tropaeolum ringspot (17, 18, 19) viruses are aphid transmissible, and thus differ from

ARSV in that respect. Insufficient data are available concerning tulip white streak virus (19). The writer found no virus listed by Holmes (10) identical with ARSV. The Annulaceae, Marmoraceae, and Lethaceae were considered especially.

Certain cucumber mosaic virus strains, including the pepper ringspot strain (7), induce ringspot symptoms in tobacco. Ainsworth's tulip virus (1) was identified as a cucumber mosaic virus strain because the latter partially protected tobacco from the former, although the tulip isolate caused only local symptoms in cucumber. His lily strain of cucumber mosaic virus (1) sometimes induced systemic mottle in tobacco but invaded cucumber systemically only once. It was identified as a cucumber mosaic virus strain by immunological studies in zinnia. Smith's lily ringspot virus (19), although aphid-transmissible, displayed no relationship to cucumber mosaic virus in immunological studies in tobacco.

ARSV appears unrelated to cucumber mosaic virus in its immunological relationships, its failure to be transmitted by aphids, and its failure to invade cucumber systemically. It resembles cucumber mosaic virus in its host range, its symptoms on several hosts (and notably in its reactions on the 2 tested cowpea strains), its distribution in the field, and its thermal inactivation point. The latter is identical with that reported by Wellman (24) for *Marmor cucumeris commelinae*. However, ARSV cannot be considered a cucumber mosaic virus strain on the basis of present evidence. Ainsworth (1) states that all cucumber mosaic virus strains he obtained from dicotyledonous hosts resembled the type. Conceivably, ARSV may have been derived from cucumber mosaic virus in a monocotyledonous host. Possibly, aphid transmission of ARSV can be obtained if suitable plant species are used under suitable environmental conditions. Serological studies are most likely to settle the question of the possible relationship between ARSV and cucumber mosaic virus.

According to Holmes (10), members of the Annulaceae incite ringspot diseases. Plants subsequently recover from obvious symptoms and display a nonsterile type of immunity. The vectors are unknown. Members of the Marmoraceae cause mosaic diseases and tend to be aphid transmissible. ARSV induces ringspot diseases from which plants sometimes recover and no virus proved recoverable from the recovered plants. Its vectors are unknown. ARSV is placed in the Annulaceae. Proof of aphid transmissibility or of a strain relationship to cucumber mosaic virus would transfer it to the Marmoraceae.

The important natural sources of ARSV have not been discovered. *Commelina* spp. and various ornamental perennials and bulbs are suspected. Townsend and Felix (22) and Felix (8) reported ringspot, ring mosaic, or oakleaf diseases of pepper or celery from the Everglades area. Townsend and Felix (22) stated that Wellman confused the celery disease with southern celery mosaic. Stoner (personal communication) has not observed this disease in the Everglades recently. Pepper is the only plant reportedly affected by the ring and oakleaf disease from the Everglades which is also known to be attacked by ARSV in central Florida. Since ARSV did not invade celery systemically and natural infection of celery by ARSV was not found at Sanford, it is unlikely that Wellman confused ARSV with southern celery mosaic on this host, at least. Relatively fine chlorotic line patterns are caused by certain strains of cucumber mosaic virus from Florida in pepper and celery, but these symptoms are distinct from those of ARSV. Whether or not Townsend and Felix worked with ARSV is unknown. Experiment station reports from the Everglades area indicate that *Commelina* spp. carry more than 1 virus, and field observations in central Florida support this contention (4), although proof has not been obtained.

Technical description. Annulus wellmanii sp. nov. **Common name:** Aster ringspot virus. **Origin:** *Callistephus chinensis* from Tampa, Florida, January, 1952. **Host range:** Fifteen plant species, including tobacco, cucumber, cowpea, nasturtium, and pansy. **Differential symptoms:** Ringspot disease in tobacco; local chlorotic and necrotic rings and spots in cucumber; broad yellow ring, line, and oakleaf patterns in pepper; cucumber mosaic virus-type lesions in blackeye cowpea; local and systemic symptoms in Sill and Walker's cowpea strain. **Transmission:** Transmitted by sap. Aphids suspected vectors, but tests negative. Properties (determined in tobacco): Thermal inactivation point, between 75 and 80°C. Immunological reactions: Negative with *Annulus tabaci, A. dubius, Marmor cucumeris commelinae*, and *M. cucumeris judicis* in tobacco. Negative with *M. cucumeris judicis* in zinnia.

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ABSORPTION OF THIOURACIL BY TOBACCO LEAF TISSUE AND INHIBITION OF TOBACCO MOSAIC VIRUS MULTIPLICATION[1]

Carl W. Nichols

SUMMARY

Leaf disks from tobacco leaves inoculated with tobacco mosaic virus (TMV) were floated on aqueous solutions of thiouracil (1,2,4,6,8,10,50, and 100 mg/l). The solutions were renewed every 24 hours. The amount of chemical absorbed by the leaf disks each 24-hour period and the amount of TMV in the leaf disks at the end of 6 days was determined. The rate of thiouracil absorption was about 50 per cent slower the second 24-hour period than the first at all concentrations. Slightly less than the original rate of absorption was resumed during the third and succeeding 24-hour periods. A 10-fold increase in concentration of thiouracil induced a 6-fold increase in amount of chemical absorbed throughout the range of concentrations studied. An absorption by 16 14-mm. disks of 0.013 mg. per 24 hours was the lowest absorption that produced maximum virus inhibition. In the range of absorption reported the inhibition of virus multiplication increased with an increased absorption of thiouracil but was not directly proportional to absorption or concentration.

Inhibition of tobacco mosaic virus (TMV) multiplication by thiouracil was reported by Commoner and Mercer (1, 2), Mercer et. al. (3), and Nichols (4). Nichols reported that a 10-fold increase in concentration of thiouracil brought about a 5-fold increase in chemical absorbed in 24 hours by tobacco leaf disks. It is the purpose of this paper to report amounts of chemical taken up during each 24 hours over a 6-day period, and the effect of the absorbed thiouracil on virus multiplication over a range of concentrations which give partial to almost complete inhibition of TMV multiplication.

METHODS.—TMV infected tobacco (*Nicotiana tabacum* L.) leaf disks were floated in continuous light (115 ft. candles) on aqueous solutions of thiouracil at concentrations of 1,2,4,6,8,10,50, and 100 mg/l for 6 days. The temperature was maintained at about 22°C. The disks were obtained, floated, and assayed for virus content according to the previously described (4,5,6) spectrophotometric method involving hot water bath and ultracentrifugation steps. The amount of recovered virus is reported as mg. of virus per 16 leaf disks.

The amount of thiouracil taken up by leaf disks over a 24-hour period was determined by the spectrophotometric method described earlier (4). Before solutions were changed at the end of each 24-hour period, samples of thiouracil solution were obtained for spectrophotometric assay. Careful washing of the leaf disks and petri dishes with distilled water at the start of the experiment and before each liquid change were necessary for best results when determining thiouracil concentrations.

RESULTS.—It was found that a drop in amount of chemical absorbed occurred at all concentrations during the second 24-hour period (Fig. 1). This was a drop of about 50 per cent. In all cases, during the third 24-hour period, more chemical was absorbed than during the second period. The amount absorbed in the third and succeeding 24-hour periods did not quite equal that for the first. After the third 24-hour period the amount of chemical absorbed *each* 24 hours was roughly constant for a given concentration. In the range of concentrations studied, the percentage of

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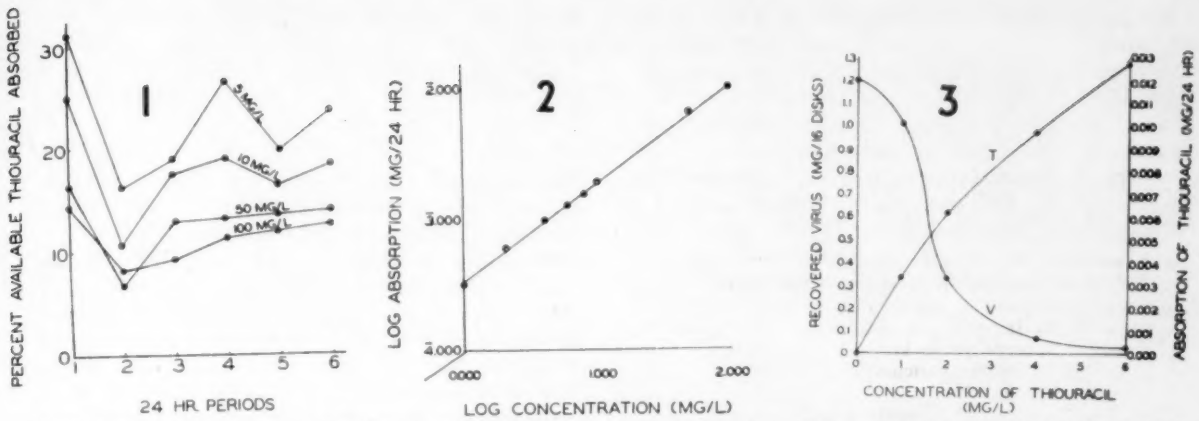

FIGS. 1–3. Fig. 1. Average percentage of available chemical absorbed each 24-hour period by 16 leaf disks floated on 10 ml. samples of thiouracil at concentrations of 5, 10, 50 and 100 mg/l.—Fig. 2. Relationship between concentration of thiouracil and amount of chemical absorbed per 24 hours by 16 leaf disks floated on 10 ml. of the solutions.—Fig. 3. Mg. of recovered virus per 16 leaf disks floated on aqueous solutions of thiouracil for 6 days (V), and average weight of thiouracil absorbed by 16 leaf disks per 24 hours (T).

available thiouracil absorbed increased with a decrease in outside concentration.

It was reported earlier (4) that a 10-fold increase in concentration of thiouracil produced a 5-fold increase in amount of chemical absorbed by 16 leaf disks floated on 10 cc. samples of the solutions for 24 hours. In the work reported in this paper, a 10-fold increase in concentration was usually associated with a 6-fold increase in chemical absorption. This was true throughout the range of concentrations tested. Not only was it true for the first 24-hour period, but it was also true when the average absorption per 24 hours over the complete 6-day period was determined for each concentration. This is a relationship which results in a straight line when the logarithm of the concentration is plotted against the logarithm of the chemical absorbed (Fig. 2).

The smallest amount of absorbed thiouracil that caused almost complete inhibition of virus multiplication was 0.013 mg. of thiouracil per 24 hrs. (Fig. 3). The region where a given increase in amount absorbed caused the greatest increase in inhibition of virus multiplication was from 0.003 to 0.01 mg. of thiouracil absorbed per 24 hours. Above and below this range a given amount absorbed was less effective in inhibiting virus. The inhibition of virus multiplication was not proportional to either the concentration of the available chemical or the amount of chemical absorbed (Fig. 3).

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DISTRIBUTION AND NATURE OF FUSARIUM RESISTANCE IN THE TOMATO PLANT [1]

R. P. Scheffer and J. C. Walker

SUMMARY

Tomato cuttings of susceptible varieties inoculated by allowing transpiration pull to take up suspensions of *Fusarium* bud cells were found to develop typical disease symptoms. Isolations immediately after inoculation and at intervals thereafter showed the fungus to be distributed throughout the stems from inoculation time until the plants collapsed. New roots developing from infected stems were always free of the fungus. Type A-resistant tomatoes, when inoculated the same way, showed some mild disease symptoms, but the plants always recovered. Isolations showed that the fungus gradually disappeared from the upper stem when plants were growing favorably. Under conditions favoring disease, the fungus lasted longer in the upper stem. Since the plants showed resistance even though the roots were free of the fungus, it was concluded that resistance was present in stems as well as in roots. Histological evidence supported this conclusion.

No disease symptoms resulted when tomato cuttings were inoculated with forms of *F. oxysporum* other than f. *lycopersici*. Such organisms survived as long as 35 days in the stem bases, but tended to disappear from the upper stems somewhat sooner than did f. *lycopersici* in type A-resistant plants.

Inoculated Type A resistant cuttings showed typical disease symptoms when they were allowed to take up dilute ethanol solutions. Uninoculated cuttings treated with the same solutions showed no visible effects. The possibility that resistance depends on continuous metabolic reactions is discussed.

The literature contains conflicting conclusions on the location of resistance to wilt (*Fusarium oxysporum* f. *lycopersici* (Sacc.) Snyder and Hansen) in the tomato plant as well as on the physiological basis of such resistance. May (13) and Heinze and Andrus (9), after grafting the tops of susceptible tomatoes to the roots of resistant varieties and vice-versa, concluded that resistance is localized in the roots. Snyder et al. (15) disagreed, at least in part, with these conclusions. The concept of the physiological nature of resistance has been still more confusing. The most recent and most specific conclusion is that tomatin, an antibiotic found in highest concentration in the leaves, is responsible for Fusarium wilt resistance (10). Recent work by Kern (11) showed that tomatin probably does not account for the Fusarium resistance of certain tomato varieties. In the present work, rather definite conclusions are presented regarding the distribution of resistance in the host plant, and a general approach toward a study of the physiological nature of resistance is indicated.

Two genetic types of resistance occur. The first of these, designated herein as type B, is found in older resistant varieties represented by Marglobe. The second type, designated herein as type A, is controlled by a single gene pair as shown by Bohn and Tucker (2), and is found in such varieties as Pan America and Jefferson. In type A plants there is little or no invasion of the vascular system of the host. In type B plants invasion is similar to that of susceptible plants and under very favorable conditions of temperature and host nutrition the disease develops, although more slowly than in a susceptible variety such as Bonny Best (4, 16).

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This investigation began as a study of the location of resistance in type A and in type B plants. From this research certain hypotheses were evolved concerning the nature of resistance, and the work was expanded accordingly.

MATERIALS AND METHODS. — Tomato plants were grown in the greenhouse in clean sand watered with mineral nutrient solutions. Most experiments required stem cuttings 6–8 in. long. These were taken from plants 5 to 7 weeks old by making a cut at an angle with a razor blade under water.

A highly virulent (R5-6) and a weakly virulent strain (A15-8) of *F. oxysporum* f. *lycopersici* and 3 forms of *F. oxysporum* which were nonpathogenic to tomato (f. *conglutinans* (Wr.) Snyder & Hansen, f. *nicotianae* (J. Johnson) Snyder & Hansen, and *F. oxysporum* Schl.) were used. Inoculum was produced by growing the fungi in Czapek's solution in shake cultures at 25°C. for 3 to 7 days. Under these conditions most of the fungus growth was in the form of bud cells which resembled microconidia in size and shape. Mycelial fragments and larger fungus aggregates were removed from the inoculum by filtering through coarse filter paper (Reve Angel, No. 202) with suction in a Büchner funnel. Filter paper of this grade allowed the individual bud cells to pass through without the mycelial fragments. In order to remove possible toxic materials from the inoculum, the bud cells were washed in saline solution or tap water by 2 centrifugations. The cells were then resuspended in tap water and adusted to the desired concentration with the aid of a hemocytometer. Unless otherwise stated, 50,000 cells per ml. were used as inoculum.

Tomato cuttings were inoculated by placing the cut ends in the bud cell suspension and allowing the cuttings to take up the desired dose of fungus cells by transpirational pull, a method which has also been

used by Keyworth (12) in a study of wilt of tomato incited by *Verticillium albo-atrum* Reinke & Berth. The usual dose of 5 to 10 ml. of the cell suspension was absorbed by the cuttings in 5 to 9 hours under conditions favoring moderate transpiration. In most cases the cuttings were kept under artificial light at 24° to 27°C. and approximately 50 per cent relative humidity. After the inoculum had been absorbed the cuttings were removed from the spore suspensions, the basal ends which had been immersed in the inoculum were trimmed, and the cuttings were replaced in a mineral nutrient solution where they were left for several days until roots formed. They were then potted in sand and placed under conditions favoring disease development. In some cases cuttings were kept in a moist chamber (with light) until roots formed. Care was taken to prevent exposures of the cuttings to direct sunlight until they were well rooted.

Disease severity was determined by rating plants for both wilt and vascular discoloration symptoms. Three wilt readings were taken as the disease progressed, and after the final reading the stem epidermis and cortex were peeled back to observe vascular browning. Wilt was rated by the method of Gallegly and Walker (5). Vascular browning was rated on a 0–4 scale as follows: 0 = no browning; 1 = browning in basal 2 in. of stem only; 2 = browning of most bundles in lower half of stem; 3 = same as 2, except that browning extended through at least ⅔ of stem; 4 = all vessels browned from base to tip of stem.

EXPERIMENTAL RESULTS.—*Disease development in inoculated cuttings.*—Preliminary experiments were conducted to standardize the procedure. Bonny Best (susceptible) cuttings inoculated as described above usually showed the first disease symptoms in 10 to 15 days. All the typical symptoms developed, including petiole epinasty, leaf yellowing, wilting and rootlet stimulation along the stems. There was considerable variation in disease development between plants and between sets of plants inoculated at different times. An attempt was made to determine whether these differences could be traced to the inoculum or to the condition of the cuttings.

Inoculum concentrations of 5,000, 50,000, 100,000, 300,000 and 500,000 cells per ml. were used and the effects on disease development determined. Ten Bonny Best cuttings, carefully selected for uniformity in size and appearance, were used for each concentration of inoculum. Results of 2 such experiments showed no significant differences in disease development. In a third experiment, 1 set of 10 cuttings was allowed to take up an average of 40,000 fungus cells per plant (assuming spores were absorbed in the same ratio as the fluid taken up), another set was given 380,000 and a third set took up 2½ million cells per plant. There were no significant differences in disease development. Apparently the lowest dose was above the minimum required for infection. These results should be compared with those of Haymaker (8) who poured spore suspensions around the roots of seedlings and found that 700,000 spores per ml. of a virulent fungus strain were required to cause infection.

The age and degree of hardness of plants were considered as possible factors in disease development. Wilt development in 10 inoculated cuttings from 60-day-old potted plants, with first flower buds, was compared with wilt in 10 inoculated cuttings from 35-day-old plants. Three weeks after inoculation, the younger plants had an average wilt index of 56, as against 4.0 for the 60-day-old cuttings. In this case, however, the younger plants were rather succulent, while the older plants were dark green and hardened in appearance. In another experiment, wilt development in 10 uniformly succulent cuttings, cut from plants grown in a warm greenhouse (not below 26°C.), was compared with wilt development in 10 more hardened cuttings of the same age from plants grown under cooler night temperatures (approx. 17°). Three weeks after inoculation the harder plants had an average wilt index of 38 as compared with an average index of 56 for the more succulent cuttings.

From these experiments it was concluded that cell concentration of the inoculum, within a very wide range, did not affect development of disease in cuttings. Nevertheless, the standard inoculum used in the following experiments was 50,000 cells per ml., and the same suspension was used for all plants in any 1 experiment. Much of the variability within experiments and between experiments apparently was due to the condition of the plants. In further work, therefore, in any 1 experiment plants of the same age were carefully selected for uniformity of size and growth habit. By using such precautions more consistent results were obtained.

The degree of resistance possessed by various tomato varieties is well known, but plants of several varieties were evaluated in order to determine whether they would fall into the same classes when inoculated by the cutting technique. For any 1 experiment, at least 10 plants per variety were inoculated, and each variety was used in at least 2 experiments. Plants were carefully selected for uniformity, inoculated with a standard cell suspension, and otherwise handled as described above. The following varieties were used. Type A resistant: Jefferson, Pan America, Southland, and 2 selections, 5-S-46M and 6-S-60M, originally obtained from Dr. C. M. Tucker; type B resistant: Marglobe, Michigan State Forcing, Rutgers and Pritchard; susceptible: Bonny Best, Indiana Baltimore, and Stokesdale. Disease symptoms, including wilt, vascular browning and leaf epinasty, appeared in all varieties, both resistant and susceptible. The resistant varieties, however, generally showed less severe initial symptoms, invariably recovered and grew normally, whereas the susceptible plants became progressively more severely diseased. Figure 1 shows results from representative experiments. There was some variation from one experiment to another, but in all experiments, the same pattern was observed, with the susceptible varieties giving the highest disease indices, type B resistant varieties somewhat lower indices, and type A

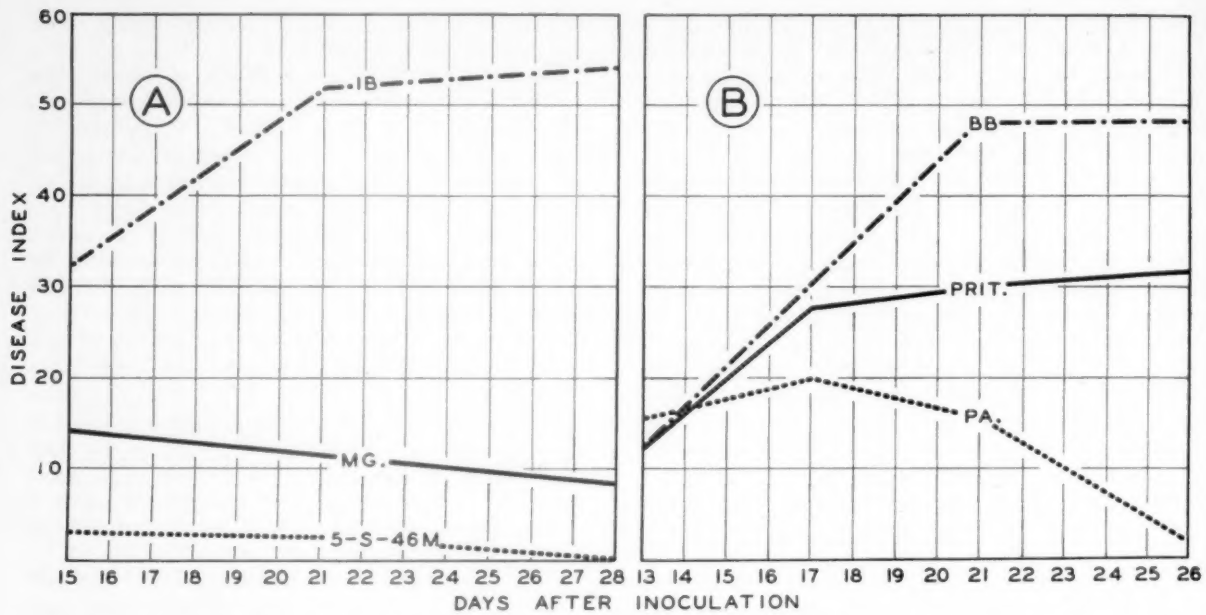

FIG. 1.—Disease development in tomato cuttings inoculated with *Fusarium* bud cells. A) Indiana Baltimore, susceptible (IB), Marglobe, type B resistant (MG); 5-S-46M, type A resistant. B) Bonny Best, susceptible (BB); Pritchard, type B resistant (PRIT); Pan America, type A resistant (PA).

resistant varieties showing initial symptoms but recovering. The same varietal classes for degrees of resistance and susceptibility held with 3 spore concentrations (10,000, 48,000 and 96,000 cells per ml.) of the inoculum.

Comparison of cutting inoculation with root inoculation.—The varieties used in cutting inoculation experiments were also inoculated by the standard root-dip procedure for a comparison of the 2 methods under the same conditions. For the root inoculations, plants were removed from the pots, the roots were dipped in a spore and mycelium suspension of *F. oxysporum* f. *lycopersici*, strain R5-6, and then reset in sand. Disease ratings taken 3 and 4 weeks after inoculation showed that the varieties had the same relative degrees of resistance whether inoculated by root dip or through cut stem ends (Table 1).

TABLE 1.—*Average wilt in plants inoculated through cut stem ends as compared with plants inoculated through roots. Values are the averages of 10 plants each*

Tomato variety	Wilt index: Root inoc. Expt. 1[a] (4-29-52)	Root inoc. Expt. 2[b] (7-9-52)	Cutting inoc. Expt. 2[b] (7-9-52)
Type A resistant varieties			
5-S-46M	0		
6-S-60M	0		
Jefferson		0	0
Pan America		0	0
Southland		0	4.0
Type B resistant varieties			
Marglobe	24		
Rutgers	18		
Michigan State Forcing	46		
Pritchard		18	20
Susceptible varieties			
Indiana Baltimore	80		
Stokesdale		42	32
Bonny Best		52	48

[a] Disease rated 31 days after inoculation.
[b] Disease rated 22 days after inoculation.

Response of cuttings to strains of the tomato wilt fungus.—Two strains of *F. oxysporum* f. *lycopersici*, R5-6 (highly virulent) and A15-8 (weakly virulent), were used to inoculate Jefferson, Marglobe and Bonny Best cuttings. Ten cuttings of each variety were inoculated with R5-6 and 10 with A15-8. The cuttings were rooted in sand and handled in the usual way. Results in Table 2 show that A15-8 had no effect on the Jefferson or the Marglobe plants, and had only a mild effect on Bonny Best. Strain R5-6 affected both Bonny Best and Marglobe, but had no significant effect on Jefferson. The experiment was repeated with essentially the same results, which agree with data obtained by others using root inoculations.

TABLE 2.—*Wilt indices for cuttings inoculated with weakly virulent (A15-8) and highly virulent (R5-6) strains of the tomato wilt Fusarium. Values are the average disease rating of 10 plants each taken 20 days after inoculation*

Variety	A15-8	R5-6
Bonny Best (Susceptible)	6.0	58
Marglobe (Type B resistant)	0	48
Jefferson (Type A resistant)	0	0

TABLE 3.—*Survival of various fusaria in inoculated tomato cuttings, as determined by isolations*

Species of Fusarium	Tomato variety	Stem position [a]	No. cuttings used each time	No. of cuttings containing *Fusarium* after— (0 days)	10 days	20 days	35 days	Disease symptoms at 35 days
F. oxysporum f. *lycopersici*, strain R5-6	Bonny Best	base	5	5	5	5	5	severe wilt
		center	5	5	5	5	5	
		top	5	3	5	5	5	
Do.	Jefferson	base	5	5	5	5	5	None
		center	5	5	4	3	0	
		top	5	4	2	0	0	
F. oxysporum f. *conglutinans*	Bonny Best	base	5	5	5	5	5	None
		center	5	5	5	0	0	
		top	5	4	1	0	0	
F. oxysporum (brome root-rot organism)	Bonny Best	base	5	5	5	5	5	None
		center	5	5	4	0	0	
		top	5	3	3	0	0	

[a] Top leaf marked at time of inoculation. All isolations were below this point.

Effect of other Fusaria on tomato cuttings.—If resistance to Fusarium wilt in tomato is limited to the root area, other forms of *Fusarium* might be expected to cause disease if introduced directly into the vascular system. The importance in the disease syndrome of extracellular enzymes (6) which are produced by many fusaria, offers support for this reasoning. Therefore, Bonny Best tomato cuttings were inoculated with bud cells of the cabbage yellows organism (*F. oxysporum* f. *conglutinans*), the tobacco wilt fungus (*F. oxysporum* f. *nicotianae*) and a brome root rotting organism (*F. oxysporum*). Ten cuttings were inoculated with each fungus, using at the same time 10 cuttings inoculated with f. *lycopersici* as controls. The experiment was repeated twice. The cuttings were rooted in sand and held under conditions favoring disease. In no case did disease symptoms appear in plants inoculated with the several forms of *Fusarium* other than f. *lycopersici*.

Distribution and survival of various fusaria in tomato cuttings.—The distribution and survival of fusaria in inoculated cuttings were determined for several reasons. First, it became of interest to determine the distribution of inoculum by the transpiration stream. Then, since resistant cuttings always recovered, an effort was made to see if the plant was freeing itself of the parasite, or whether it was merely tolerating the fungus in the vascular bundles. The latter consideration also applied to nonpathogenic forms of *Fusarium* which were introduced into the vascular system of tomato plants. Distribution and survival were determined by a simple isolation procedure. Slices from all parts of the stems were cut immediately after inoculation and at intervals thereafter, surface sterilized, and plated on potato dextrose agar to determine the presence or absence of the fungus.

The distribution and survival of the highly pathogenic strain R5-6 in susceptible Bonny Best cuttings were determined first. Twenty cuttings, each 6 in. long, were taken from 36-day-old plants and inoculated with bud-cell suspensions in the usual manner. The cuttings were allowed to take up the inoculum for 8 hours, and slices from various parts of the stems of 5 plants were plated. The top leaves of the remaining cuttings were marked, and the cuttings were rooted in sand under conditions favoring disease development. Isolations were made from 5 plants at 10, 20 and 35 days after inoculation, respectively. In each case, isolations were made from the top (5 in. from the cut base, just below the marked leaf), the middle (3 in. from the base), and the bottom of the stem (1 in. from the cut end). Isolations from the newly formed rootlets were attempted at 10 and 20 days after inoculation. The pathogenicity of reisolated fusaria was tested by inoculating young Bonny Best plants by the root-dip method. The experiment was repeated 2 times with slight variations in treatment and procedure.

Results in Table 3 show that the pathogenic fungus was present immediately after inoculation in the basal and central parts of all cuttings, and in the tops of most cuttings as well. In other experiments with larger cuttings the fungus has been isolated from sections as far as 7 in. up the stems immediately after inoculation, and in many cases from the bases of leaf petioles. At 10, 20 and 35 days after inoculation, fungus was reisolated from all parts of the stems (Table 3). Disease symptoms usually appeared in 10 to 15 days, and plants were often completely killed by the 35th day. *Fusarium* could be recovered from almost all the petioles of wilting leaves, and, in the same plants, from at least half of the nonwilting leaves. In no case was the pathogen recovered from roots, even after many attempts.

The pattern of survival of the highly virulent strain, R5-6, in type A resistant cuttings (var. Jefferson) was somewhat different. Immediately after inoculation, the fungus was as well distributed in the resistant cuttings as in Bonny Best cuttings (Table 3). Twenty days later, however, the fungus appeared to be disappearing from the upper parts of the stems, and after 35 days was found only in the stem bases. In contrast to

TABLE 4.—*Effect of shading inoculated Jefferson (resistant) plants on the survival of Fusarium as determined by isolations*

Treatment and date	Position on stem assayed [a]	No. of cuttings used	No. of cuttings containing Fusarium at days after inoculation indicated—			
			7 days	10 days	20 days	35 days
Shaded Sept. 1952	base	5			5	5
	center	5			5	5
	top	5			1	1
Unshaded (Feb. 1953)	base	5		5	5	5
	center	5		4	3	0
	top	5		2	0	0
Unshaded (June, July, 1953)	base	10	10		10	10
	center	10	10		10	4
	top	10	9		4	0
Shaded (June, July, 1953)	base	10	10		10	10
	center	10	10		10	10
	top	10	10		7	8

[a] Top leaf marked at time of inoculation. All isolations were made below this point.

the pattern in the susceptible variety, in no case was the fungus reisolated from the new top growth that occurred after inoculation. As in the susceptible variety, however, the fungus could not be isolated from the roots which developed after inoculation. This fact is considered especially significant, since the resistant plants do not show typical disease development, yet contain the highly pathogenic fungus in the stems but not in the roots. It is thus apparent that resistance is not confined to the roots, but occurs throughout the stem as well. Other workers have reached this conclusion after inoculating above-ground parts of intact plants, but the difficulty and uncertainty of obtaining diseased plants by such methods have made their conclusions less convincing.

Effect of shading on survival of Fusarium in resistant plants.—The survival of strain R5-6 in resistant plants (var. Jefferson) growing under favorable conditions of light and temperature was compared with the survival in resistant plants which were shaded. Cuttings were inoculated as usual by the bud-cell technique, and rooted in sand. One group (30 plants) was shaded with cheesecloth and kept in a greenhouse where the temperature was not below 24° C. Another set of 30 plants was not shaded and was kept in another section of the greenhouse where the temperature occasionally went as low as 20° C. at night. Results of isolations from these 2 sets of plants are summarized in Table 4. After 35 days the fungus was largely restricted to the stem base in unshaded plants, while in shaded plants, which were more succulent and spindly, it was found in the middle stem of all plants, and in the top in 8 out of 10 plants. As usual, none of these plants were showing disease symptoms at the end of the experiment.

Survival of nonpathogenic fusaria in tomato stems.—Isolation experiments were also carried out with tomato cuttings (var. Bonny Best) which had been inoculated with bud cells of forms of *Fusarium* other than *F. oxysporum* f. *lycopersici*. The cabbage yellows fungus (f. *conglutinans*) and a brome root-rot *Fusarium* were used. Immediately after inoculation and after 10 days these Fusaria were well distributed in the cuttings (Table 3). After 20 days, however, they were found only in the stem bases. Thus these forms of *F. oxysporum* appeared to be even less well adapted to wilt-susceptible tomato than f. *lycopersici* was to the wilt-resistant variety.

Anatomical response of Fusarium-inoculated resistant and susceptible cuttings.—Jefferson and Bonny Best tomato cuttings were inoculated and rooted in sand. After 25 days, when the resistant plants had recovered fully and the susceptible plants were showing wilt symptoms, sections of stems were fixed in formalin-alcohol-acetic acid mixture, embedded in paraffin, and 15μ sections were cut and stained with safranin and fast green. Examination of stained sections showed in the Jefferson (resistant) stems a small amount of vascular browning. This was, however, confined to the primary xylem which was present at the time of inoculation. Secondary xylem developing around infected bundles after inoculation was free of the fungus. Many but not all bundles examined had much proliferation of the xylem parenchyma around infected vessels, as shown in Fig. 2. In susceptible Bonny Best stems the fungus spread, in most cases, into the new secondary xylem. The tendency for xylem parenchyma around infected vessels to proliferate was much less evident than in the resistant cuttings. Judging from these sections, the fungus in the resistant cuttings appeared to be held in check enough for the host to retain a growth advantage.

Effect of alcohol on expression of resistance.—Potato tubers treated with ethanol and other alcohols have been found by Behr (1) to lose temporarily the capacity to produce periderm under wounds. Tubers of resistant varieties treated thus were also found to be temporarily susceptible to *Phytophthora* and *Fusarium* rots. Similar experiments were carried out with tomato stems to determine the effect of alcohol on Fusarium wilt resistance.

Resistant tomato cuttings (var. Jefferson) were in-

oculated with strain R5-6 bud cells, then transferred into mineral nutrient solution which contained ethanol. Five cuttings each were left for 7 days in solutions containing 1.0, 0.5, and 0.2 per cent alcohol, respectively. Inoculated and uninoculated controls, and uninoculated, alcohol-treated controls were used. The treated controls showed no visible effects of alcohol, except that root formation was retarded; they grew well after removal from the alcohol. Inoculated treated cuttings at all 3 alcohol concentrations showed vascular browning and collapsed with wilt after 12 to 15 days. In many cases the fungus grew out of the stem surface at the bases of the cuttings. The experiment was repeated several times with similar results. In another experiment, inoculated lots of 5 cuttings each were exposed to 0.5 per cent ethanol solutions for 3, 6, and 9 days, respectively. The appropriate inoculated and uninoculated controls were included. Fifteen days after inoculation the wilt indices for the 3-, 5-, and 9-day plants were 35, 65, and 75 respectively. Vascular browning rated on a scale of 0 to 4 averaged 1.0, 1.2, and 2.2, respectively, for the 3 exposure groups. The inoculated, untreated controls had no wilt and an average browning of 0.5, while the uninoculated, treated controls had no wilt or browning. The inoculated cuttings exposed to alcohol for 3 days were showing signs of recovery at 15 days, but the 6- and 9-day exposed plants showed no signs of recovery.

Methyl, isopropyl, and N-butyl alcohols were also tested for their effects on resistance. Butanol at concentrations of 0.5, 1.0, and 2.0 per cent was toxic and caused cuttings to collapse. Isopropyl alcohol caused little injury in 0.5 and 1.0 per cent solutions, but when inoculated cuttings were treated for 7 days there was no detectable increase in susceptibility. Methanol was used in concentrations up to 2.0 per cent, which caused little injury to cuttings. However, inoculated cuttings treated with 1.0 and 2.0 per cent methanol showed no significant increase in disease as compared to inoculated, untreated controls.

Discussion. — An inoculation technique has been used in these experiments which eliminates the roots as a factor in infection. Tomato cuttings were allowed to take up suspensions of *Fusarium* bud cells directly with the transpiration stream, after which the cuttings were rooted in sand. Susceptible tomato varieties inoculated in this way with *F. oxysporum* f. *lycopersici* became diseased. Several lines of evidence indicated that the typical syndrome of Fusarium wilt was exhibited. Leaf yellowing, petiole epinasty and wilt symptoms were all observed, and the incubation period was only slightly shorter than that in root-inoculated plants. Type A resistant, type B resistant, and susceptible varieties inoculated by the root dip and by the cutting methods showed the same relative degrees of resistance to Fusarium wilt. Highly and weakly virulent strains of the wilt *Fusarium* had the same relative effects on tomato whether inoculated through cut stems or by root dip. There is thus little doubt that the typical disease syndrome was being observed in plants inoculated through cut stem ends with bud-cell suspensions.

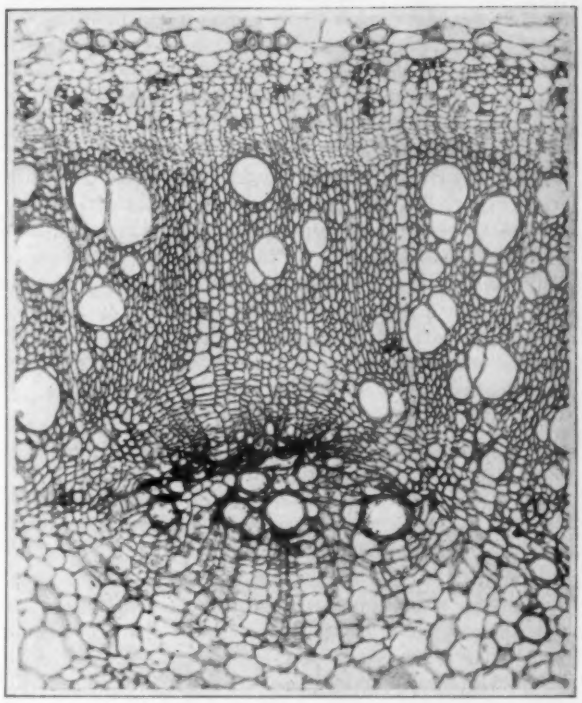

Fig. 2.—Cross section of stem of Jefferson (type A resistant) tomato 25 days after being inoculated as a cutting with bud cells of the tomato wilt *Fusarium*. Note browning and mycelium in older vessels. New secondary xylem is free of the fungus.

There was 1 important difference between root-inoculated and cutting-inoculated plants. With root inoculations, vascular pathogens must invade and grow through the roots before the critical stem tissue is infected. The pathogen can therefore be isolated from the roots, and in many cases has been observed to cause damage to such roots. When stems were inoculated through cut ends, on the other hand, the new roots which grew from the infected stems were always free of the fungus. By this method plants were obtained which exhibited typical disease symptoms in the tops, but which had root systems free of the parasite.

Previous workers (9, 13) have concluded that resistance to *Fusarium* in tomato is localized in the roots. Evidence for this conclusion was obtained by grafting resistant scions to susceptible roots and vice-versa. When the susceptible-root, resistant-top combination was inoculated, wilt developed; whereas with the resistant-root, susceptible-top combination, few symptoms became evident. If the localized resistance theory is correct, then inoculated cuttings of resistant varieties should develop disease, since there are no roots to act as barriers to the pathogen. Inoculated cuttings of several highly resistant varieties did show limited symptoms from which they soon recovered and grew normally. Since the fungus survived for a long

time in the stems, and since it was not present in the roots, the only conclusion available is that the resistance factor is present throughout the stem, and probably in the roots as well. The importance of vascular plugging as a cause of wilting in diseased plants (14) offers a new explanation for the data obtained in grafting experiments. When resistant roots were used with susceptible tops, the fungus was probably largely excluded from the susceptible tops, but when resistant tops were used with susceptible roots, the fungus probably caused vessel blocking below the graft and wilt was therefore expressed in the resistant tops. Also, copious growth of the fungus below the graft might provide metabolic products to which the resistant tops are sensitive, and which may bring about plugging.

Histological work gave further indication that resistance is present in the stem. In susceptible cuttings, the fungus effectively invaded all xylem tissue, while in resistant cuttings *Fusarium* did not spread to the new secondary xylem tissue, and the host plant was able to function normally as soon as new conductive tissue was developed around the affected area. There was also evidence of proliferation of the xylem parenchyma cells around infected vessels, a condition which was much less evident in susceptible varieties.

The results discussed so far do not eliminate tomatin (10) as the factor responsible for resistance. Several considerations, however, make this theory seem implausible. Tomato varieties which are resistant to *Fusarium* are not necessarily resistant to *Pseudomonas solanacearum* E.F.Sm. and vice-versa, although the 2 organisms affect the same host tissues and do not differ greatly in sensitivity to tomatin *in vitro*. Tomatin content in susceptible and resistant plants is about the same, but resistant plants are said to be able to maintain this same level after inoculation, while in susceptible plants the tomatin content falls. This fact, however, does not prove that tomatin is responsible for resistance. It may indicate only that tomatin content is reduced with systemic infection, which is rare in resistant plants and is the usual case with susceptible plants. If the fungus were equally distributed in resistant and susceptible plants, and the resistant plants were still able to maintain their tomatin while susceptible plants lost it, then the theory would appear more convincing. Recently Kern (11) has determined the tomatin concentration in roots, stems, and vascular fluids of tomato. He found tomatin in these critical areas to be much too low to inhibit spore germination or mycelial growth of Fusarium. Tomatin thus appears to be inadequate as an explanation of resistance.

A theory of resistance to root and vascular wilt diseases which has had recent support is the "root microflora" idea (3, 7). The flora on the roots of resistant varieties supposedly is antagonistic to the pathogen in question, while susceptible varieties have very little of the antibiotic-producing flora. Attempts have been made to correlate such factors with resistance to Fusarium wilt of banana (7). Results of cutting inoculations of resistant tomatoes obviously eliminate root microflora as a possible explanation of resistance to Fusarium wilt of tomato.

Data on the survival of *Fusarium* in inoculated cuttings gave no positive information on tomatin, but did indicate another possible approach to the resistance problem. Immediately after cuttings took up bud cells with the transpiration stream, isolations have shown the fungus to be well distributed in the stems of both resistant and susceptible varieties. Isolations from stems at 10, 20, and 35 days after inoculation showed that in susceptible varieties the fungus remained throughout the plant, if environal conditions were right, until the plant collapsed. In resistant plants, the pathogen was still well distributed after 10 days, but after 20 days it had lost ground, and at 35 days was restricted to the stem base. This relative disadvantage of the fungus was affected by the vigor of the resistant host plant. When inoculated, rooted resistant cuttings were shaded and kept at a temperature favorable to disease, *Fusarium* was still well distributed in the stems after 35 days, even though no disease symptoms were evident. Such results possibly indicate that an understanding of resistance should be sought in a study of metabolic reactions of resistant and susceptible plants.

The survival of several forms of *Fusarium* in cuttings was determined to compare type A resistance to f. *lycopersici* with tomato resistance to other forms of *Fusarium*. In no cases did other forms of F. *oxysporum* (f. *conglutinans*, f. *nicotianae*, and a brome root-rot form) cause disease symptoms when introduced directly into the vascular system. Form *lycopersici* (strain R5-6) did cause a temporary but definite response in Type A resistant plants, especially when predisposing and environal factors favored rapid disease development. Isolations showed that f. *conglutinans* and the brome form survived for some time in Bonny Best stems but generally did not survive as well as f. *lycopersici* did in Type A resistant cuttings. There may be a qualitative difference between Type A resistance to f. *lycopersici* and the resistance of tomato to other Fusaria, although these experiments offer no definite proof of this.

Further understanding of the nature of resistance was sought by treating inoculated cuttings with low concentrations of alcohol. When inoculated Type A resistant tomato cuttings were allowed to take up 0.2 and 0.5 per cent solutions of ethanol, typical Fusarium wilt symptoms developed. Since the appropriate uninoculated, alcohol-treated controls showed no such effects, the results are considered to reflect a decreased resistance to the disease. Alcohol is said to interfere with respiration, and also to decrease the permeability of cell membranes. In either case metabolic reactions in general may be reduced. Although the relation of host metabolism to disease resistance is difficult to interpret, it is possible that resistance is dependent upon continuous metabolic activity.

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INFLUENCE OF ACIDIC BUFFER SPRAYS ON INFECTION OF TOMATO LEAVES BY ALTERNARIA SOLANI [1]

Curt Leben

SUMMARY

In greenhouse tests with *Alternaria solani* on tomato foliage, acidic buffers applied as protectant sprays gave 53–79 per cent disease control. *A. solani*, as well as some other plant pathogenic fungi, were found to be sensitive to acidic conditions in *in vitro* tests. The reactions of tomato leaf surfaces that had been sprayed with an acidic buffer were usually more acid than those of leaves sprayed with water.

In studies with the antibiotic helixin B, it was observed that the pH of the spray had a marked effect on the protectant action of the antibiotic in the early blight foliage test with tomato. The antibiotic was carried in 0.01 M potassium phosphate buffer, at pH 3, 4.7, or 6; included in the experiment for comparative purposes were control sprays of buffers without the antibiotic. Results showed that the antibiotic was less effective under acidic conditions, as has been reported (17), and that the pH of the spray influenced the degree of infection even though no antibiotic was present. This last observation led to a more extended study of the effect of acidic buffer sprays on the incidence of tomato leaf infection by the early blight pathogen, *Alternaria solani* (Ell. & Mart.) Jones and Grout, and to allied studies, which are the subjects of this paper. A short account of some of the work has already appeared (16).

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Materials and methods.—In early blight infection tests young Bonny Best tomato plants were sprayed with a test buffer, dried, and then sprayed with a spore suspension of the pathogen. After a period of incubation, the incidence of disease was noted. Plants 25-35 cm. high were used, and there were 3-11 replicate plants for each treatment in each test. Test solutions and spore suspensions were sprayed to the point of runoff on the upper side of 3 (or in some tests, 4) leaves of each plant. Since none of the test materials seemed to possess surface active properties, the amount of liquid on the leaves was approximately the same in all treatments. Test solutions were usually dried on the plant 4-6 hours before inoculation with a spore suspension of *A. solani* containing 50,000 to 100,000 spores per ml. For all tests described in this paper an isolate of *A. solani* requiring no special treatment for the production of spores was used; spores were derived from potato-glucose agar [2] plate or tube cultures that had been incubated for 7-14 days at 20-24° C. Inoculated plants were incubated for 2 days in a moist chamber (22-26°) and then placed in a greenhouse at

[2] Per l.: extract from 200 gm. of sliced, peeled potatoes steamed 0.5 hour in 500 ml. of water; glucose, 25 gm.; agar, 17 gm.

aproximately 24°. The lesion number was recorded in 1-4 days, depending on the conditions for the development of the disease. All data were analyzed statistically.

Hydrogen-ion determinations were made with a glass electrode, line-operated, Beckman pH meter. In making measurements on hardened agar, an equal volume of water was mixed well with the agar, and readings were made after about 0.5 hour. For pH determination with small volumes, the reference electrode was modified so that the KCl orifice was in a small glass tube adjacent to the glass electrode; the sample was held in a small cup made from the end of a test tube. This modified electrode unit permitted determinations on 0.1 ml.

Phosphate buffer solutions of a desired pH were made by mixing 0.01 M H_2PO_4 with 0.01 M monobasic or dibasic sodium or potassium phosphate. The H_2SO_4 + Na_2SO_4 buffer system was made by mixing 0.05 M solutions of H_2SO_4 and NaOH.

Conventional slide germination tests were made using 0.01 M potassium phosphate buffers at different pH levels as the circumambient fluid for *A. solani* spores. Slides were incubated at 26° in Petri dish moist chambers, and germination was noted in 4 drops after 18-24 hours. Spores were considered germinated if germ tubes were longer than the spore. The pH levels were 3, 4, 5, 6, 7, 8, and 8.6. A water control (pH 6-6.4) was also included in each test. In 1 experiment the pH of the pooled drops was determined at the end of the germination period, and the drift during this time was no more than 0.3 unit from the initial pH.

The effect of pH on the growth of various fungi was studied using potato agar [3] adjusted to different pH levels. After the medium had been autoclaved and cooled to ca. 45°, it was adjusted to the desired pH by adding sterile 1 N H_2SO_4 or NaOH. Plates were poured, the agar allowed to harden, and the pH of the medium (the initial pH) determined at the time of seeding. Petri plates containing 20 ml. of medium and Felsen dishes containing 4 ml. in each quadrant were used. Seeding was by means of streaks of spore suspensions, which were made from potato-glucose agar[2] cultures incubated at 26° for 7–14 days. In each Petri dish there were 4 streaks, and in each Felsen plate quadrant, 1 streak was used. After 40–48 hours the pH drift of unseeded media was no more than 0.2 unit from the initial pH, except in the medium with an initial pH of 8.6, which changed to pH 8.

In the "leaf stroke" method of determining pH at the leaf surface, inoculated plants were removed from the moist chamber and dried. Approximately 0.2 ml. of water was placed in a small area on the sprayed side of a leaflet, and the modified electrode unit was dipped into the water and moved gently over the leaflet surface. On removal of the unit sufficient liquid remained between the electrodes for a determination.

[3] Per l.: extract from 40 gm. of sliced, peeled potatoes steamed 0.5 hour in 500 ml. of water; glucose, 5 gm.; agar, 17 gm.

TABLE 1.—*Effect of potassium phosphate buffer sprays at different pH levels on lesion production by A. solani on tomato leaves*

Spray	Average lesions per plant[a]			
	Test 1[b]	Test 2[c]	Test 3[d]	Test 4[d]
Potassium phosphate buffer, pH 3	46	116	67	340
Do. do. do. pH 6	165	223	----	----
Water	155	245	144	1038

[a] Three plants used in each treatment in tests 1 and 2, 11 in test 3, and 10 in test 4. Tests made at different times.
[b] MSD at the 0.01 point is 94.
[c] MSD at the 0.05 point is 103.
[d] Differences significant at the 0.01 point, as indicated by the "t" test.

RESULTS.—*Early blight tests.*—In the studies with helixin, 5 tests contributed data that indicated an influence of pH on disease incidence. In each test the trend was the same: there were fewer lesions on plants that had been sprayed with the potassium phosphate buffer at pH 3 than on those sprayed with the potassium phosphate buffer at pH 6 or with water. A comparison over the 5 tests between the pH 3 buffer treatment and the water treatment indicated the difference to be highly significant. On the other hand, in the 3 experiments in which the buffer at pH 6 was included, analyses showed that no significant difference between this treatment and the water treatment existed. Example data from 2 of the experiments are given in Table 1, tests 1 and 2. Later, 2 additional tests with potassium phosphate buffers were made. Data from these experiments are also given in Table 1 (tests 3 and 4).

Two additional infection tests were made with sodium as well as potassium phosphate buffers to extend the earlier studies and to note a possible influence of the cation. Buffers at pH 3 and 6 were used, and a water spray was provided as a control. In both experiments buffer sprays at pH 3 containing either cation resulted in highly significant disease control. There was no significant difference between the water and the sodium buffer at pH 6 treatments in either test. There was likewise no significant difference between the potassium buffer at pH 6 and the water treatments in 1 test; in the other, however, the difference was significant, a result that can probably best be attributed to chance factors. It was concluded from these experiments that there was no preferential relationship between the 2 cations, as has been noted elsewhere (21).

Work with the phosphate buffers suggested that either the low pH or the action of phoshate at the low pH was responsible for the reduced lesion numbers. It therefore seemed desirable to try acidic buffers other than the phosphates. It was also desirable to try to find more efficient buffers, since at the outset of the

work it was realized that the buffering capacity of phosphate was poor over the range of interest, pH 3 to 5. A number of weak organic acids (e.g., citric, succinic, and tartaric) with more suitable pKa values were applied as test sprays at a concentration of 0.05 M and at pH 3. These preparations produced small necrotic spots on leaves after the 2-day period in the moist chamber, even though plants had not been inoculated with *A. solani*. This of course precluded the use of these substances in infection studies. An effort was made to find a suitable inorganic buffer. The first system tried, H_2SO_4 + Na_2SO_4, proved nonphytotoxic and, although titration tests showed that the buffering capacity of this material from pH 3 to 5 was essentially the same as the potassium phosphate system, it was decided to make exploratory experiments with this combination without further search for a more desirable substance.

Four infection tests were made with the H_2SO_4 + Na_2SO_4 system. Data are given in Table 2. In all of the tests the acidic sprays were effective in reducing the disease. No phytotoxic effects were noted.

Effect of pH on germination and growth of A. solani.—From the foregoing work it was concluded that low pH at the surface of the leaf was the main if not sole cause of disease control by the acidic buffers. One of the possible means whereby the acid acts is the inhibition of the germination and growth of *A. solani*. To examine this point a number of tests were made on the influence of pH on the germination of spores in liquid and on agar, and on the growth of the fungus on agar.

Three slide germination tests were made using potassium phosphate buffers at different pH levels. The results in all were parallel. At pH 3, germination was 2–5 per cent; it was over 90 per cent at pH 4 through 8.6 and in the water control. A better assessment of the influence of pH was obtained by measurement of the length of germ tubes. At pH 4 tubes were 0.4–0.6 mm. long, at pH 5 they were 0.5–1 mm. long, at pH 6 through 8 and in the water control they were 1.5–3 mm. long, and at pH 8.6 the length was 0.5–1 mm. Three additional germination tests over approximately the same pH range were made using potato agar adjusted to different pH levels: in each test the results were similar to those obtained in the slide tests.

For studies on the effect of pH on the growth of *A. solani* on agar, seeding streaks of suspensions containing 100,000–150,000 spores per ml. were employed. There were 3 tests using Petri plates and 3 with Felsen dishes. Results obtained with acidic media in Felsen dishes are given in Table 3: it will be noted that the growth of *A. solani* was inhibited at pH 3.2 and 4.2. The results in the Petri dish tests were the same except that inhibition was also noted at pH 5.1 (a rating of "3"). The absence of inhibition at this pH

TABLE 2.—*Effect of H_2SO_4 + Na_2SO_4 buffer sprays on lesion production by A. solani on tomato leaves*

Spray	Average lesions per plant[a]			
	Test 1[b]	Test 2[b]	Test 3[c]	Test 4[c]
H_2SO_4 + Na_2SO_4 buffer, pH 3	190	112	127	292
Na_2SO_4 solution, pH 6[d]	----	----	643	733
Water	485	301	609	687

[a] In tests 1 and 2 there were 9 replicate plants for each treatment, in test 3, 7 replicates, and in test 4, 8 replicates. Tests made at different times.

[b] Differences significant at the 0.01 point, as indicated by the "t" test.

[c] MSD at the 0.01 point for test 3 is 263; for test 4 it is 218.

[d] At pH 6, H_2SO_4 is completely ionized, and the spray was therefore a Na_2SO_4 solution with no buffering capacity.

TABLE 3.—*Growth of various fungi on potato agar at different pH levels*

Organism	Growth[a] on media with the initial pH[b] of:			
	3.2	4.1	5.1	6.0
Alternaria solani (Ell. & Mart.) Jones & Grout	0	2	4	4
Cladosporium cucumerinum Ell. & Arth.	0–1	1–2	4	4
Colletotrichum circinans (Berk.) Vogl.	0	3–4	4	4
C. lagenarium Ell. & Halst.[c]	0–1	1–4	4	4
C. lindemuthianum (Sacc. & Magn.) Briosi & Cav.	1	2–4	4	4
C. phomoides (Sacc.) Chester	1	2–3	4	4
Endoconidiophora fagacearum Bretz[c]	0	1–2	4	4
Fusarium oxysporum f. *lycopersici* (Sacc.) S. & H.	2	3–4	4	4
Glomerella cingulata (Stonem.) Spauld. & v. Schrenk	1	4	4	4
Helminthosporium sativum P. K. & B.	1	2–3	4	4
H. turcicum Pass.[c]	1–3	3–4	4	4
Mycosphaerella pinoides (Berk. & Blox.) Stone	1	3–4	4	4
Stemphylium sarcinaeformiae (Cav.) Wilts.	0	2–3	4	4
Venturia inaequalis (Cke.) Wint. (Four isolates.)[c]	0	1–2	2–4	4
Verticillium albo-atrum Reinke & Berth.	0	2–3	4	4

[a] Visual estimation: 0=no growth, 4=maximum growth. Data after 60 hours at 26° and based on 3 experiments. Range of response over the different tests is indicated where hyphenated entries appear. Data for *V. inaequalis* after 10 days at 16°.

[b] Initial pH varied from figure given by no more than 0.1 unit in any given test.

[c] Results based on 2 tests.

TABLE 4.—*The pH of leaf surfaces sprayed with 0.01 M potassium buffer at pH 3 or with water*

Test number	pH of buffer-sprayed plants after[a]:			pH of water-sprayed plants after[a]:		
	1-2 hours	24 hours	48 hours	1-2 hours	24 hours	48 hours
1	----	5.7–8.1	----	----	7.5–8.1	----
2	4.2–5.1	4.9–6.2	5.6–6.3	6.0–8.1	7.1–8.8	7.6–8.5
3	3.8–5.6	6.0–6.6	----	6.0–6.6	7.2–8.8	----
4	4.9–6.2	6.9–7.3	----	6.4–6.8	7.1–7.8	----

[a] In test 1, each entry represents the range of 3–6 determinations each on a different leaf; in tests 2, 3, and 4 the range is for 5–6 determinations. In experiment 1 the test and control leaves were on different plants; in tests 2, 3, and 4 they were on the same plants. Tests made at different times.

level in tests with Felsen dishes was probably due to the fact that in this vessel the fungus was able to neutralize the acid faster than in the Petri dishes, which contained more medium.

It was concluded from these experiments that germination and growth of the isolate of *A. solani* used in these tests was inhibited under conditions more acid than pH 5.

The pH at the leaf surface.—The foregoing sections have reported studies which suggest that a low pH is responsible for the disease reduction resulting from the treatment of leaves with acidic buffers, and that *A. solani* is especially sensitive to acidic conditions. It was of some interest, therefore, to try to determine the pH of the surface of the leaf after treatment with an acidic buffer. For these tests plants were sprayed with water or with a 0.01 M potassium phosphate buffer at pH 3, inoculated, and placed in the moist chamber in the usual manner. Early tests with pooled inoculum drops (individual drops were usually too small for separate determinations) showed that while samples from the buffer-sprayed plants usually gave lower pH values than samples from water-sprayed leaves, the variation indicated the need of an additional method of testing. To serve this end the "leaf stroke" method was devised.

Data for 4 tests using the leaf stroke method are given in Table 4. In each the trend is the same; leaves that had been sprayed with the buffer were usually more acid than those that had been sprayed with water.

The pH of inoculum drops was also estimated by means of indicator paper ("Hydrion Papers", Micro Essential Laboratory, Brooklyn 10, N. Y.). The reaction range on individual leaves 24 hours after inoculation was usually indicated to be pH 3–5.5 on buffer-sprayed plants and 6–8.8 on plants sprayed with water.

On the basis of these experiments it is concluded that the acidic buffer spray materially lowered the pH at the leaf surface, but that this effect did not persist long. All 3 methods of pH determination indicated that there were areas of varying pH on the buffer-sprayed leaves. This variation might be expected on the basis of an uneven deposit of the buffer; that such areas appeared to exist on leaves sprayed with water is surprising. More precise methods for making pH determinations on individual inoculum drops are needed, since the pooling of several drops is not deemed satisfactory, and the leaf stroke method gives an "average" pH of areas covered. Indicator papers are not believed to be sufficiently accurate for precise determinations. Thus, the results do not permit final conclusions, but they strongly suggest that some areas of buffer-sprayed leaves were sufficiently acid for a long enough time to reduce the germination and growth of *A. solani*, which, as has been noted, is especially sensitive to acid.

Effect of the pH of the medium on growth of other plant pathogenic fungi.—In view of the inhibition of *A. solani* under relatively weak acid conditions, it became of interest to examine the effect of acidic pH levels on the growth of other plant pathogenic fungi. Tests were made in Felsen dishes using potato agar adjusted to various pH levels. The organisms studied and the results of the tests are given in Table 3.

These data indicate that a number of plant pathogenic fungi in addition to *A. solani* are sensitive to acidic conditions. Of especial interest are *E. fagacearum* and *V. inaequalis*.

DISCUSSION.—Hydrogen-ion concentration has been demonstrated to have an influence on the course of a number of plant diseases. The suitable adjustment of the pH of the soil has been used for many years for the control of diseases incited by certain soil-borne plant pathogens. In the experiments described by Yarwood (30), it seems likely that the acidic conditions on the leaf were responsible for the therapy of certain powdery mildews by sulfuric, nitric, and acetic acids. The injection of basic substances into elm trees has been associated with the lessening of symptoms of the Dutch elm disease (9,10), and the work of Blood (6) indicates that acid is responsible for the effectiveness of the citric and lactic acid and pulp steeping treatments of seed for the control of bacterial canker of tomato. In the present work it has been shown that when acidic buffer sprays were applied to tomato foliage prior to inoculation with *A. solani*, reductions of 53–79 per cent in the early blight disease resulted. It was also demonstrated that acidic conditions inhibited the germination and growth of *A. solani*, and the evidence strongly suggests that acidic conditions on the leaf were responsible for the lessening of the disease through inhibition of the fungus.

The present work may be thought of as an effort to understand one of the fundamental factors that may

influence the action of plant sprays and dusts. From the available data it is not likely that the acidic buffers used in these studies, as such, would be suitable for the effective control of tomato early blight (or perhaps other diseases), since these materials appear to have a number of limitations. The buffering effect is short lived, probably because of some type of neutralization of the acid by the host, and the rate of neutralization may well vary from host to host or under differing conditions. Complete control is to be desired, and this was not attained with the early blight disease. An additional limitation is the likelihood of these buffers being washed from the plant by meteoric water. However, further research may uncover buffers without some of these limitations. For reasons that will be mentioned, it seems likely that if buffers are to find use in the chemical control of plant diseases, they will be employed primarily as addenda in spray and dust formulations.

On the basis of the present studies some degree of control of the pH at the plant surface would seem feasible through the use of buffers. One might expect that the buffering capacity of the plant would quickly neutralize an acidic substance applied to the plant surface, but the present work indicates that this opinion may not be correct, especially since the buffering capacity of the substances used was not great in the pH range of interest. Should it be possible to control the pH effectively, information now available suggests that this procedure would be desirable from several points of view. In the first place, the present paper has indicated that pH control at the plant surface may be useful in reducing disease by maintaining conditions unfavorable for growth or infection by the pathogen. This idea was put forth by Moons (19), although he presented no experimental evidence to substantiate his view. It would be of value to extend the present investigation to other acid sensitive fungi that have been noted and to the plant pathogenic bacteria. The effects of basic buffers have not been considered in this work, but this is also an avenue for additional research.

Secondly, it is desirable to control the pH at the leaf surface to obtain the maximum inhibitory action of toxicants used for disease control. The fact that pH influences the activity of bactericides was well known by 1920 (20), and in 1922 Young (31) noted that in spore germination tests the fungitoxic activity of various types of sulfur was markedly influenced by pH. Since then others (1,2,3,12,13,14,15,22,23,24, 25,27) have found that pH influences the inhibitory action of many compounds on fungi. Smith, Walker, and Hooker (27) suggested that the phenomenon may be of practical importance, particularly with acidic compounds. As far as is known, the influence of pH on toxicant action has not been demonstrated in plant tests except with helixin (17). It is possible by interpolation of the early blight dosage-response curve to show that approximately 3 times as much of this antibiotic is required for 95 per cent disease control when the material is applied in a buffer at pH 3 as when it is applied at pH 6. Thus, the effect of pH may be expected to be considerable with certain compounds, especially with those that have the properties of weak acids or bases, as do many antibiotics (5,7). With weak acids or bases pH governs the degree of ionization, and where differences exist between the biological activity of the ions and the corresponding undissociated molecule, as have been reported in all cases investigated, a small pH change may have a great effect on the activity, or upon the availability of a suitable receptor. Albert has reviewed this subject, especially in relation to the action of drugs, in a series of lectures (29). The effect of pH on the biological activity of most weak acids and bases has been generalized recently (26); in some instances, it may be possible to alter a compound so that pH does not influence its action (4).

The third reason for controlling the pH is that pH may have an effect on performance factors of a material applied for disease control, aside from the effect on activity of the toxicant as such. These factors include coverage, adhesion, stability, penetration of the host, and phytotoxicity. For example, Brown and Hoskins (8) demonstrated that the pH of the spray was important in relation to the deposit of oil on a standard surface, and Garman (11) found that pH influenced the adhesion of a bordeaux-flotation sulfur mixture. The pH of a spray has been shown to affect the droplet charge and the charge of the surface on which it falls (28). Helixin (17) and antimycin A-102 (18) were more resistant to washing from the leaf by simulated rain when the deposit was acidic than when it was more alkaline.

These various effects of pH on toxicant performance probably do not act independently, and additive or nullifying interactions may be expected. An example of interaction may be taken from the research on helixin (17). If this material is formulated with a buffer at pH 3 and used as a protectant for the control of early blight of tomato, the deposit would be resistant to the washing action of water, and protection could be expected through the action of the acidic buffer in addition to that afforded by the antibiotic. On the other hand, acidic conditions would not permit the maximum toxicity of the antibiotic to *A. solani*. The total efficiency, then, depends on the various types and degrees of interactions.

The information now available suggests that more attention should be given to pH and buffering capacity in the assessment of toxicants. With the early blight protectant test, for example, the results may be influenced profoundly by variation in the pH of the test spray. Both the position and slope of the dosage-response curve may be altered. For example, in early blight tests with helixin (17) approximately the same level of disease control was obtained with each of a series of 2-fold dilutions of the material. Subsequent study showed that the more dilute preparations were more alkaline than the concentrated preparations and

that helixin was more effective under these more alkaline conditions. On the adjustment of the pH of the different dilutions to a common level, a more conventional dosage-response curve was obtained. In the various types of *in vitro* tests for the assessment of chemicals for plant disease control one may also expect spurious results unless the pH is controlled.

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PHYTOPATHOLOGICAL NOTES

A Quick Method of Preparing Semipermanent Slide Mounts of Fungi on Agar.[1] W. E. SACKSTON. In the course of studies on the sequence of spore germination and colony development of *Septoria linicola* (Speg). Gar., the pathogen of the pasmo disease of flax, a quick, simple method was devised for preparing semipermanent slide mounts of spores and mycelium *in situ* on agar from cultures in petri dishes.

To prepare a mount by this method, a block of agar bearing the desired material is cut out of the petri dish and placed on a clean microscope slide. The block is then trimmed so that it is about 4–6 mm. smaller in diameter than the cover slip to be used on it. If the

[1] Contribution No. 1311 from the Botany and Plant Pathology Division, Science Service, Department of Agriculture, Ottawa, Canada.

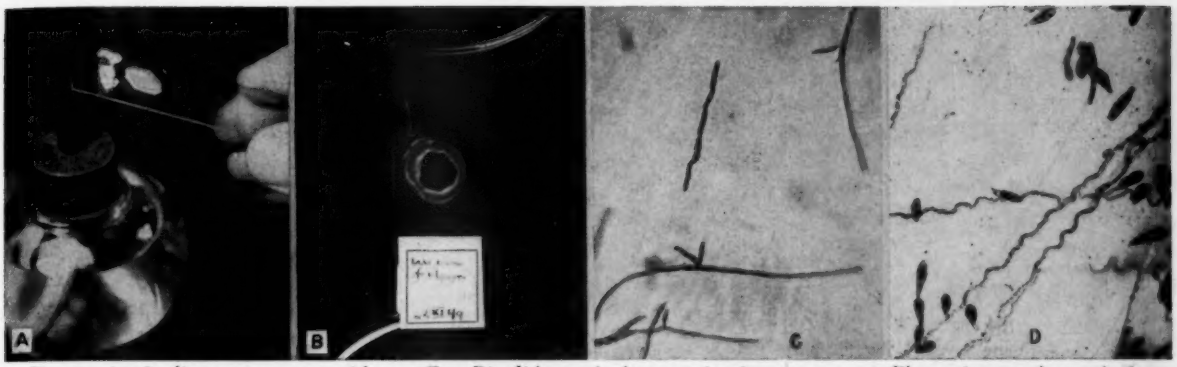

FIG. 1—A) Sealing agar mount with paraffin; B) slide ready for examination or storage. Photomicrographs made from stored slides: C) germinating spores of *Septoria linicola* after 3 months' storage; D) conidia and mycelium of *Alternaria* sp. after 2¾ years' storage.

block is too large, the ring of sealing material will be thin and the agar will dry out too rapidly; if the block is too small, its area may be insufficient to support the cover slip in position. The shape of the block is not important, so long as no corners project too near the edge of the cover slip. Round cover slips give the best results.

The cover slip may be placed directly on the agar block, or a small drop of water or lactophenol mounting medium, with or without stain, may be added. If liquid is used, the amount should be just sufficient to extend to the edge of the agar block. After the cover slip is placed on the agar, the slide should be checked under the microscope. If the mount is satisfactory, it is ready to be sealed.

The preparation is sealed with paraffin. "Tissuemat," a commercial paraffin imbedding material, has proved very satisfactory. A few chips of paraffin, each about 5 mm. in size, are placed on the slide, resting against the edge of the cover slip. The slide is held at a slight angle from the horizontal and its upper end is warmed gently in a flame (Fig. 1, A).

The slide should not be heated excessively, or the agar will melt. When the paraffin melts, it spreads under the cover slip and encircles the agar block. Additional chips of paraffin may be melted if necessary to bring the paraffin seal to the edge of the cover slip. When the paraffin cools, it forms an opaque white ring around the agar block under the cover slip (Fig. 1, B). The slide is checked again to make sure that the mount is satisfactory, labelled, and stored until it can be studied. If the specimen is damaged in mounting or sealing, another slide can be prepared and sealed in 1 or 2 minutes.

Slides of *S. linicola* spores and mycelium prepared by this method in March, 1948, and stored in a desk drawer, were studied and photographed 3 months later (Fig. 1, C). Spore measurements made 7 months after the slides were prepared were similar to those determined on fresh material. The agar blocks in many of the slides made in 1948 were dry when the slides were checked in June, 1953. Individual slides that were still in good condition, however, included preparations of pycnidiospores of *S. linicola* and of *Darluca filum* (Biv.-Bern.) Cast., mounted in March, 1948, and in November, 1949, respectively; conidia and mycelium of *Helminthosporium sativum* P. K. & B., and *Alternaria* sp. (Fig. 1, D), mounted in July, 1948, and in September, 1950, respectively; and urediospores of *Puccinia helianthi* Schw., conidia of *Erysiphe cichoracearum* DC., and pollen grains of *Helianthus annuus* L., mounted in September, 1948.

The method described above cannot be expected to produce permanent slides. It does, however, make it possible to prepare quickly and easily slides of fungus spores or mycelium growing on agar in petri dishes, or mounted on agar blocks to avoid the movement often encountered in liquid preparations, and to keep such slides for 6 months or longer.—Plant Pathology Laboratory, Winnipeg, Manitoba, Canada.

Relationship of Foliage to Stimulation of Adventitious Roots in Crown Gall.[1] W. C. PRICE AND C. GAINOR. Galls incited by *Agrobacterium tumefaciens* (E. F. Sm. & Towns.) Conn are associated with stimulation of adventitious roots in certain host plants[2,3,4] such as *Kalanchoë daigremontiana* Hamet & Perrier. Experiments of de Ropp[4] indicated that a root stimulating substance originates in gall tissue on sunflower and other hosts. Locke, Riker, and Duggar[3] suggested that a substance normally originating in expanding foliage passes downward to gall tissue and there increases proliferation, and also that a growth substance originating in gall tissue passes downward.

In testing virulence of a transplant of our $B6_1$ cul-

[1] Contribution from the Plant Virus Laboratory, University of Pittsburgh.

[2] Brown, Nellie A. 1929. The tendency of the crown-gall organism to produce roots in conjunction with tumors. Jour. Agr. Res. [U. S.] 39: 747-766.

[3] Locke, S. B., A. J. Riker, and B. M. Duggar. 1938. Growth substance and the development of crown gall. Jour. Agr. Res. [U. S.] 57: 21-39.

[4] deRopp, R. S. 1947. The growth-promoting and tumefacient factors of bacteria-free crown-gall tumor tissue. Am. Jour. Bot. 34: 248-261.

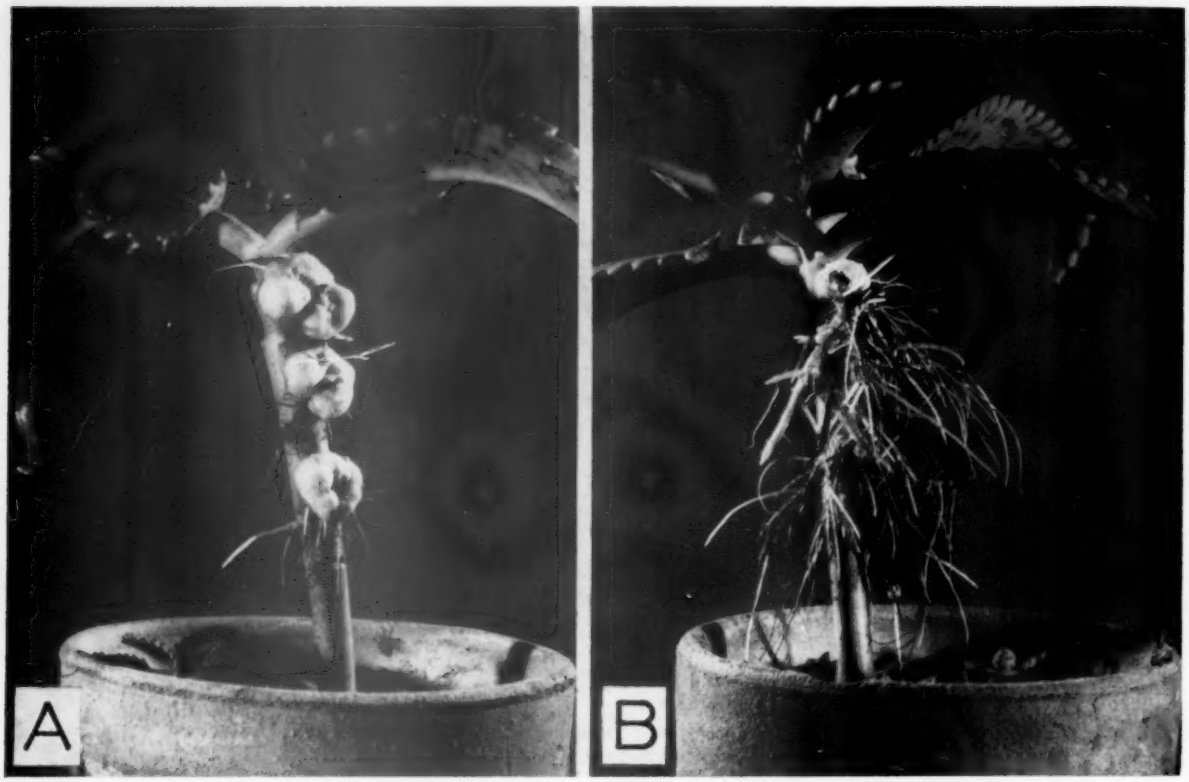

FIG. 1. Plants of *Kalanchoe daigremontiana* 2 months after inoculation with *Agrobacterium tumefaciens*. A) A plant on which all but a single pair of terminal leaves were removed. B) A plant on which 3 pairs of basal leaves were removed and terminal leaves allowed to develop normally; gall hidden by roots were comparable in size to those on the plants in A). (Photographs by R. B. Gordon)

ture[5] of *A. tumefaciens*, we removed a few of the upper leaves on plants of *K. daigremontiana* to facilitate needle-puncture inoculation; subsequently, the extent of production of adventitious roots was roughly proportional to the number of leaves remaining. This suggested the possibility that the growth substance responsible for root stimulation was organized in leaves, rather than in gall tissues, and then transported to the gall sites. To test this hypothesis, inoculations were made into *Kalanchoë* plants in various states of artificial defoliation and at various times over a 2-year period. Three needle-puncture inoculations with a 24-hour old culture of *A. tumefaciens* were made into the stem of each plant at ¾ in. intervals, beginning ½ in. from the tip. Plants were 4–15 in. tall when inoculated.

When all leaves but the pair at the extreme tip were removed and the new growth kept trimmed back, plants frequently died; on those that survived, galls smaller than usual developed, but there was little stimulation of adventitious roots. When the pair of small leaves at the terminal was allowed to expand fully and removed only after the next pair above had reached ½ mature size, the galls were as large as, or larger than, those on plants with all leaves intact (Fig. 1,A); adventitious roots grew out from below the galls but soon withered away. In contrast, plants with several pairs of leaves above the points of inoculation generally developed an abundance of adventitious roots (Fig. 1,B); roots grew from tissues just below the galls and continued development for 2 months or more. Plants with the largest number of leaves above the gall sites usually produced roots most abundantly. When leaves at the base of the plant were left intact but those above the lowest inoculation site removed, very few adventitious roots were stimulated. After the galls on defoliated plants had grown for about 6 weeks, growth of new leaves at the apex did not stimulate growth of adventitious roots.

When all leaves except the terminal pair were removed from *Kalanchoë* plants 0, 1, 3, 6, 8, 10, 13 or 15 days after stems were inoculated with *A. tumefaciens*, the galls produced were equal in size to those on control plants, but few roots grew from the gall sites. Three weeks after inoculation, terminal growth was allowed to take place normally, following which roots developed extensively but not so extensively as on control plants.

All leaves below the terminal pair were removed from 2 plants having sizable galls and extensive roots

[5] Gainor, C. and W. C. Price. 1953. Studies concerning colonial variants of Agrobacterium tumefaciens. Phytopathology 43: 509-512.

resulting from inoculation one month previously. Root development promptly ceased on the 2 plants while roots already formed withered and died. As new terminal growth was made, starting about 5 weeks after the original inoculation, root development was again stimulated so that in a month the gall sites bore extensive root systems. This was in contrast to the previous failure of roots to be stimulated when terminal leaves were again allowed to grow 6 weeks after the original defoliation.

These results suggest, but do not prove, that a substance responsible for stimulation of adventitious roots is organized in leaves and transported downward to the site of gall tissue. That this substance is not indole-3-acetic acid is suggested by the fact that 2 per cent of this material in lanolin, sufficient to stimulate an epinastic response, applied to stumps of petioles above points inoculated with *A. tumefaciens* did not stimulate development of adventitious roots in *Kalanchoë*, nor did beta-indole-butyric acid nor alpha-napthoxyacetic acid.

The failure to correlate size of gall with abundance of foliage above the gall sites, except where terminal foliage was almost lacking, plus the striking correlation of adventitious root development with abundance of foliage, leaves doubt that stimulation of adventitious roots at the gall sites is primarily dependent upon a food factor. However, the experimental evidence does not rule out the possibility that a food factor is involved.—Department of Biological Sciences, University of Pittsburgh, Pittsburgh 13, Pa.

REPORT AND ABSTRACTS OF THE 1953-54 WINTER MEETING OF THE NORTHEASTERN DIVISION OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The ninth annual winter meeting of the Northeastern Division of the American Phytopathological Society was held at West Springfield, Massachusetts on November 5 and 6, 1953. Approximately 100 persons attended.

Twenty scientific papers were presented. The evening program, arranged by E. G. Rex, was devoted to plant quarantines. George G. Becker, S. D. Whitlock, and Emil Kostal of the U. S. Bureau of Entomology and Plant Quarantine described, with illustrations, their activities at the Port of New York and in Europe. About 25 pathologists expressed interest in a trip to observe quarantine operations at the Port of New York in the spring of 1954, with Mr. Rex in charge of arrangements.

It was decided to devote the evening program at the next winter meeting to the presentation of fresh field data on fungicides and other aspects of disease control. The invitation of the Eastern States Farmers' Exchange to utilize the same facilities at West Springfield for the next annual meeting was accepted, with November 4 and 5 as the tentative dates.

Officers elected for 1954 were: *President*, J. S. Tidd; *Vice President*, A. B. Burrell; *Secretary-Treasurer*, A. C. Tarjan; *Councilor*, F. O. Holmes.

A. B. Burrell, *Secretary-Treasurer.*

ABSTRACTS OF PAPERS PRESENTED AT THE MEETING

Some facts about peach canker. Daniel F. Cohoon and Robert H. Daines. Peach canker infections incited by *Fusicoccum amygdali*, Del., occur throughout the year, with maximum numbers in spring and fall. Infections of blossoms produce blossom blight and frequently twig cankers. Infections at bud-scale scars produce a basal canker which girdles and eventually kills the new shoot. Wounds resulting from fruit removal may become infected causing cankers on the bearing wood. The leaf scars constitute important infection courts which, when infected, produce the typical nodal cankers on current season's growth. Artificial inoculation by wounding leaf scars produced infections in all varieties tested; however, in nature a considerable variation in varietal susceptibility exists. Leaf infections have been observed as early as June 10; the number of infected leaves increases as the season progresses. The symptoms expressed by infected leaves may be confused with what appears to be a physiological injury on certain varieties. Neither natural infections nor artificial inoculations have as yet shown leaf infections to be directly responsible for twig cankers. Fruit infections take place through wounds caused by bacterial spot, insects, or other means. The symptoms on fruit are similar to brown rot in early stages, later developing black, erumpent, pycnidia in the center of the rotting area.

Control of peach canker. Daines, Robert H. and Daniel F. Cohoon. Peach canker, caused by *Fusicoccum amygdali*, Del., has been so destructive to the Golden Jubilee variety that few plantings now remain. Continued culture of Jerseyland, Early East, Raritan Rose, Red Haven, Triogem, Summercrest, Hale, Bracket, Belle of Georgia, and Eclipse varieties is threatened. Experimentation has demonstrated that, although infections resulting in twig cankers may occur throughout the year, the principal infection periods occur in spring and fall. During 1951–1953, a delayed dormant application containing monocalcium arsenite 3#, oil 1½ qts., and Bordeaux mixture 4-6-100 resulted in a 96, 94, and 30 per cent reduction of spring cankers in the respective years. During 1951 and 1952, the period from delayed dormant through bloom was short whereas this period lasted 6 weeks in 1953. Injury to twigs followed the use of the monocalcium arsenite-Bordeaux spray. Ferbam 1½#, captan 2#, and Phygon 1# were applied at the delayed dormant, pink-bud and full-bloom stages during 1953. Three applications of ferbam reduced spring infections by 25–35, captan by 48–62 and Phygon by 60 per cent in 1 orchard. The use of captan, Phygon, and the carbamates during the fall (leaf-dropping period) has resulted in a significant reduction in bud cankers.

Control of Phomopsis canker of gardenia in the cutting-

bench and on mature plants. DAVIS, SPENCER H. Cuttings dipped in a spore suspension of *Phomopsis gardeniae*, dried, and immersed in solutions of PMAS (1, ½, or ¼ pint per 100 gals.) for 1 minute prior to placement in the cutting-bench remained disease-free. In a second series, non-inoculated cuttings placed in *Phomopsis*-infested sand which had been mixed with Fermate at rates of 2, 1, ½, or ¼ lbs. of the dry powder per 100 lbs. of sand yielded disease-free plants. Slight, temporary retardation of root elongation was observed at the higher concentrations. PMAS at 1, ½, or ¼ pint per 100 gal. as a sand drench was toxic to the cuttings. Three-year-old plants, inoculated near the soil line with spore suspensions or mycelial mats, received a single spray with PMAS (1 pint/100 gal.) 1 hour, 1, 2, 3, or 4 weeks after inoculation. Any single spray application within 2 weeks after inoculation gave control. Immediately after wounding the base of similar plants, a spray with Fermate (2 lbs./100 gals.) prevented infection when spore suspensions were applied up to 2 weeks after the spray application. Neither spray caused injury to the mature leaves or wood, although PMAS did cause burning of young leaves and flowers and abscission of mature buds.

Sprays for leaf spot on English hawthorn. DOCHINGER, LEON S. AND STEPHEN BACHELDER. An English hawthorn (*Crataegus oxyacantha rosea* Willd.) which is annually defoliated during late summer in New Jersey by the leaf spot organism, *Fabraea maculata* Atk., was selected for testing the effectiveness of 7 fungicides. A single branch, 2 or 3 in. in diameter, was used for each treatment. The initial spray was made on May 1, when the terminal growth of the shoots averaged 3 in. Subsequent sprays were made on May 10, 21, and June 3. Applications were made with a hydraulic sprayer using the following amounts of the respective fungicides per 100 gallons: zineb, 1½ lbs.; Phygon-XL, 1½ lbs.; Captan, 2 lbs.; Manzate, 1½ lbs.; PMAS, ½ pt.; Vancide 51, 10 pt., and Bordeaux 8-8-100. Within a week severe spray injury was observed on the branch treated with Vancide 51. To a lesser degree burning was seen on the leaves and flowers of the branches sprayed with Bordeaux and PMAS. Data taken on September 3 showed partial disease control by Captan and apparent suppression of the leaf spot disease by zineb and Manzate. New growth and flowering stimulated by early defoliation was observed during the first week of October on the untreated and treated portions of the tree except for those branches sprayed with either zineb or Manzate.

Penetration and infection following direct germination of the sporangia of Phytophthora infestans. FERRIS, VIRGINIA ROGERS AND G. C. KENT. Though it has long been known that the sporangium of *Phytophthora infestans* (Mont.) deBary is able to germinate directly by means of a germ tube rather than indirectly by means of zoospores, there has been doubt concerning the ability of this germ tube to penetrate the potato plant and initiate infection. Recent studies by the authors indicate that penetration and infection follow direct germination. Cleared and fixed whole leaflets and fresh epidermal strips were examined, as well as sections made from both fresh material and material that had been killed and fixed. In experiments in which inoculated excised leaflets were kept under conditions known to favor direct rather than indirect germination there was an abundance of directly germinated sporangia but no evidence of zoospore formation. Mycelium was found consistently throughout the tissues of these leaflets after 48–60 hours incubation. Penetration by germ tubes from sporangia was observed in a number of preparations.

Comparative performance of chemical soil treatments for control of Fusarium wilt of carnations. GASIORKIEWICZ, E. C. Fusarium wilt is one of the major diseases of carnations. Wilt resistant varieties of commercial type are not available and since the pathogen is soil borne chemical control methods were tested. Dithane Z-78, dibromobutene, Goodrite ZAC, Crag F531, natriphene and chlorobromopropene were applied as soil drenches on a bimonthly schedule. None of the materials gave any significant control over the checks. The materials used did not affect the pH of the soils or the essential elements, nitrogen, potassium, phosphorus, calcium, magnesium, aluminum, or ammonia. In soluble salt tests final readings were higher than initial readings in the Dithane Z-78 and Crag F531 treatments. Final readings on soluble salts were lower than initial readings in the Goodrite ZAC, natriphene and chlorobromopropene tests. None of the treated plants equalled the checks in the production of flowers. The bromine compounds gave the lowest yields. Natriphene and Crag F531 consistently enhanced the growth characteristics of both varieties, Hercules Virginia and Northland.

Effect of temperature on symptom expression of carnation mosaic virus on selected host plants. GASIORKIEWICZ, E. C. During November and December 1952, the effects of an isolate of carnation mosaic from Yoder's Seedling 115 transmitted mechanically were compared on selected host plants at various temperatures. All test seedlings were grown before inoculation on open greenhouse benches at 65° F. After inoculation, equal numbers of plants of *Asclepias syriaca* L. (milkweed), *Helichrysum bracteatum* Andr. (strawflower), *Gomphrena globosa* L. (globe amaranth), and *Dianthus barbatus* L. (sweet william) were held at each of the following approximately constant temperatures: 55°F., 60°F., 68°F. and 75°F. At these temperatures symptoms were not observed on milkweed or strawflower. Initial symptoms incited were similar on globe amaranth and sweet william. Chlorotic mottle appeared on *G. globosa* 2 days after inoculation at 60°F. and in 4 days at the other temperatures. There was no further symptom development on this host plant. Chlorotic mottle appeared on sweet william about 6 days after inoculation. The necrosis which followed was more marked at the higher temperatures.

A partial host range of the necrotic ring spot virus in the genus Prunus. GILMER, R. M. Bud inoculations with 3 sources of necrotic ring spot virus (Wisconsin Barnard 1-12; New York CR 5-1; New York 19B7/8-1) were made to 38 species of Prunus. Mechanical inoculations from these sources to cucumber produced apparently identical diseases. Symptoms attributable to the necrotic ring spot virus were obtained in all species tested except *P. cistena.* Source CR 5-1 produced yellows-like symptoms in one clone of *P. japonica* (purported) and symptoms of prune dwarf in Albion plum. Comparative virulence of the 3 sources was not consistent throughout the host range. Species of the genus Prunus to which the necrotic ring spot virus was transmitted and from which it was recovered include: *americana, apetala, armeniaca, avium, besseyi, bliereana, canescens, cerasifera, cerasifera* var. *pissardi, cerasus* var. Brassington, *cistena, caroliniana, communis, domestica, dropmoreana, dunbari* (purported), *fruticosa, glandulosa, ilicifolia, japonica* (purported), *laurocerasus, lyoni, maacki, mahaleb, maritima, nana, newporti, padus, persica, pensylvanica, salicina* (20 varieties), *serotina, serrulata, sieboldi, tenella, tomentosa, triloba,* and *virginiana* var. *demissa.* A virus component of source CR 5-1 was transmitted to 2 apple seedlings. Transmission of necrotic ring spot virus was achieved when inoculating buds were removed 96 hours after inoculation. Prior removal of inoculating buds resulted in failure to transmit the virus.

Effect of metabolites of Actinomycetes on pigmentation and spore formation in phytopathogens. GROSSBARD, ERNA. *Fusarium oxysporum* f. *lycopersici* failed to form a pigment on modified Czapek-Dox; however, when used as a test organism in filter paper disc assays of 4 crude, unidentified antibiotics produced by actinomycetes, pigmentation in an unusual pattern occurred. Adjoining the area of partial inhibition a pigmented circle appeared, the rest of the mycelium remaining white. On potato-dextrose the entire mycelium frequently was pigmented; however, intensification of color occurred. The same antibiotics also stimu-

lated pigmentation in *Helminthopsorium victoriae*. Most of the active antibiotics induced pigment intensification in *Colletotrichum atramentarium* on Czapek-Dox but it was weak or absent on potato-dextrose. Associated with this was an acceleration in the formation and greater density of stromata. Several metabolites accelerated formation of coremia in *Ceratostomella ulmi*, while others stimulated sporulation in *Streptomyces scabies* or sclerotia formation in *Verticillium albo-atrum* var. *dahliae*. These effects were often associated with marked antibiotic action. These observations suggest that the tested metabolites contained specific diffusable substances stimulating either pigmentation and/or formation of spore bearing and resting bodies and that these were frequently associated with antibiotics. As phytopathogens occur in nature in association with other microorganisms these stimulation effects may be of interest in the study of parasitism.

Fusicoccum leaf spot and canker of peach and control. GUBA, EMIL F. The identity of the pathogen *Fusicoccum amygdali* Del., was confirmed by comparison with specimens on almond (*Prunus amygdalus*) from France. Pathogenicity of the fungus was established by infection of the host with conidia. The fungus causes irregular dead areas in leaves and cankers about the nodes of the shoots. Infection of herbaceous shoots is through leaf axils. More serious infections in autumn and spring occur through bud scales and leaf scars. Infection of uninjured fruit has not been successful. Shoot buds may be killed but shoots sometimes develop considerable length before girdling is fatal. Pycnidia and conidia develop simultaneously with the disease in leaves and twigs; infection and fresh spore inoculum are continuous throughout the growing season. Satisfactory control of disease was obtained by 1) eradication of cankers, 2) encouragement of new growth, 3) protection of trees throughout the growing season with a fungicide, beginning at the late dormant stage. Thiocarbamates thiram, Phygon, Captan and lime sulfur are toxic to Fusicoccum conidia; wettable sulfur is unsatisfactory.

The influence of various fungicides on the chlorophyll content of apple foliage. HILBORN, M. T. AND B. E. PLUMMER, JR. Samples from fungicide-sprayed apple leaves were taken shortly after terminal bud formation from 1948 to 1953 inclusive. Chlorophyll content was then determined spectrophotometrically. A fine-particle, dry wettable sulfur spray was the control. On McIntosh foliage materials that caused visible spray injury, such as Phygon, invariably reduced chlorophyll, usually by at least 16 per cent. Other materials that caused no visible spray injury also reduced chlorophyll. Crag Fruit Fungicide 341 (glyodin) caused 4, a sulfur-Phygon mixture 7, and a sulfur-ferbam mixture 11 per cent reduction, respectively. Some dithiocarbamates increased chlorophyll, but effects were variable with materials of a similar chemical nature. Iron salts usually caused an increase, 13 per cent for Vancide and 34 per cent for ferbam. However, a tank mix of ferric sulfate and sodium dimethyl dithiocarbamate caused a 1 per cent loss. The manganese salts were erratic in behavior. Manganese Vancide caused a 21 per cent loss, while Manzate caused a 44 per cent increase in chlorophyll. The combination of thiram with sulfur increased chlorophyll 43 per cent. On Red Delicious foliage glyodin caused a 62 per cent increase in chlorophyll when compared with sulfur.

Further studies in forecasting potato late blight in Connecticut. HYRE, R. A. The theoretical prediction of late blight of potato was compared with reported occurrences for the years 1902-52. Criteria investigated for disease development were 6, 8, 10, 12, and 14 consecutive favorable days, determined by different combinations of concurrently favorable precipitation and temperature. Possibly favorable precipitation and temperature criteria investigated were: cumulated precipitation of the growing season equalling or exceeding the cumulated average; 7-day total precipitation equalling or exceeding the average 7-day total; 10-day total precipitation equalling or exceeding the average 10-day total; and, 7-day average temeprature not exceeding 75°, 77°, or 79° F., respectively. Prediction was considered successful only when predicted date preceded date of discovery. Predictions were most successful, 78 per cent, when based on periods of 8 or 10 consecutive favorable days resulting when 10-day total precipitation was favorable, and 7-day average temperature did not exceed 77° or 79° F. Criteria suggested for trial are 8 or 10 consecutive favorable days using 10-day total precipitation and 77° F. temperature. With this combination 9 of the 11 failures occurred when blight was predicted but none was recorded (though possibly present), and 2 when blight was found before being predicted. In no case was blight severe when not predicted.

A leafhopper-borne disease from Western Europe. MARAMOROSCH, KARL. During the summer of 1953 various species of cicadellid leafhoppers were collected in Holland and tested on different plants in a greenhouse in Wageningen. Near Maastricht, insects of the species *Euscelis plebejus* spp. *plebejus* (Fall.) were obtained from grass in an orchard of cherry trees infected with the Eckelrade disease. In tests with single insects caged individually on plants, 5 of 16 leafhoppers transmitted a previously unknown disease to crimson clover (*T. incarnatum*) plants. First symptoms of disease somewhat resembled those caused in crimson clover by the wound-tumor virus; deep ridges, resulting from enlarged veins, were seen on the upper surfaces of leaflets. On the lower surfaces pronounced enations were formed. Later symptoms were characterized by chlorosis progressing from the outer part towards the midrib of leaflets. Leaves on shortened petioles remained small and were malformed. This disease is one of the first leafhopper-borne virus diseases recorded from Western Europe. It is hoped that further studies will bring more information on the host range of the virus and on its possible connection with the causative agent of the Eckelrade disease.

Dissemination of spindle tuber by contaminated tractor wheels and by foliage contact with diseased potato plants. MERRIAM, DONALD AND REINER BONDE. Studies were conducted to determine reasons for recent increase in spindle tuber in Maine. They showed the disease is readily transmitted by brushing actively growing healthy plants with diseased foliage. From 80 to 100 per cent of the plants inoculated by this method became diseased. The disease also was spread by driving contaminated tractor wheels over the foliage of healthy plants. The disease spread resulting in 1 experiment was 13 per cent for Kennebec, 6 per cent for Green Mountain, and 4 per cent for Katahdin and Cobbler varieties. On the other hand the disease was not readily transmitted by aphids, grasshoppers, or flea beetles. The writers believe that the tractor-drawn machinery used in producing the crop may be important in the dissemination of spindle tuber. Tractor-drawn machinery is now almost universally used in Maine. Kennebec vines are very large. The disease is very difficult to detect in Kennebecs.

Influence of growth regulators on resistance of beans to Colletotrichum lindemuthianum. NATTI, JOHN J. AND MICHAEL SZKOLNIK. In greenhouse tests, resistance of Red Kidney bean plants to the alpha and susceptibility to the gamma strain of *Colletotrichum lindemuthianum*, and susceptibility of Kentucky Wonder plants to the alpha and resistance to the gamma strain were not altered by treatment of plants with growth regulators. Bean seedlings were grown for 8 days following emergence in sand flushed 4 times daily with nutrient solutions containing 2,4-dichlorophenoxyacetic acid at concentrations of 2.5, 0.5, and 0.1 p.p.m., alpha naphthaleneacetic acid at 5.0, 1.0, and 0.2 p.p.m., and gamma-(indole-3)-n-butyric acid at 10.0, 2.0, and 0.4 p.p.m.; and then for 2 days at twice these concentrations prior to inoculation. Red Kidney plants were infected with the gamma strain only and Ken-

tucky Wonder plants with the alpha strain. Plants of both varieties treated with the highest concentration of 2,4-D were less severely infected than were plants from other treatments. This treatment, also, suppressed growth and delayed development of the first internode and trifoliate leaf, tissues which were usually severely infected in plants from other treatments. Control provided by 2,4-D treatment was probably due to suppression of development of susceptible tissues rather than to modification in metabolism of plants induced by growth regulators.

Variability in symptoms and signs of the sclerotinia disease of potatoes on Long Island. PARTYKA, ROBERT E. AND WILLIAM F. MAI. Morphologic symptoms occur on main stalks and on secondary branches, infection frequently being observed at axils of petioles and at points of injury. Evidence of infection is characterized by water-soaked areas which become light to dark brown in color later in the season. Infections near the base of the stalk often result in death of the entire stalk. Infection occurring through senescent leaves has been observed. In 1952, symptoms were first observed in early June, and in 1953, in early July. Wet weather prevailed during the early portion of the growing seasons of both years. This was followed by exceptionally hot and dry weather in 1952; in 1953 there was a dry spell from mid-May until late June, followed by normal precipitation. In 1952, small areas were observed in which all plants were killed by the disease. In 1953, in these same areas, although a considerable number of plants were infected, only an occasional plant was killed. The most noticeable sign of the disease is the presence of sclerotia within or on the surface of infected tissue. Under moist conditions, apothecia may be observed growing from sclerotia located in the upper half-inch of soil.

An effective method for field screening of promising fungicides for the control of potato late blight. RICH, A. E., J. D. BILBRUCK AND H. V. TOKO. There is a definite place for a small-scale field evaluation of promising new fungicides intermediate between greenhouse screening and large-scale field testing. An effective method has been worked out, using potatoes and *Phytophthora infestans.* Potatoes were planted in "hills" 6 ft. apart each way, with 4 whole seed tubers in each hill. Each hill constituted a plot and treatments were replicated 4 times. The potatoes were planted near trees which provided shade part of the day. Between the trees and the plots, a border of potatoes was planted and inoculated with *Phytophthora infestans.* Infected plants from this border were dragged through the plots on rainy mornings. This method of inoculation was effective in spite of a hot, dry season. Plants were sprayed by hand with knapsack sprayers. There was a close correlation between disease control and yields.

Controlling root-knot infections of greenhouse tomatoes with 3-p-chlorophenyl-5-methyl rhodanine. TARJAN, A. C. Among a group of chemical compounds tested for their nematicidal value, 3-p-chlorophenyl-5-methyl rhodanine showed such versatility in controlling root-knot infections that it was selected for more extensive greenhouse tests. Groups of potted 5-week-old tomato plants, *Lycopersicon esculentum* hort. var. Rutgers, roots of which were infected with root-knot nematodes, *Meloidogyne incognita,* received either a foliar spray containing 10,000 p.p.m. of the chemical emulsified in water, the dry chemical incorporated into the upper inch of soil at the rate of 4 gm. per sq. ft., or a soil drench of 100 ml. of emulsified chemical at the rates of 4, 2, 1, or 0.5 gm. per sq. ft. of soil. Plants harvested 6 weeks later showed that complete control of the root-borne parasites had been accomplished only when the chemical had been applied in emulsion form at the rates of 4 and 2 gm. per sq. ft. In another test, noninfected seedlings were transplanted into soil in which the dry chemical had been mixed at the rates of 2, 1, or 0.5 gm. per sq. ft. of surface area. Each plant was then inoculated with 5 nematode egg masses. Examination of roots, 2 months later, showed that complete control had been achieved in all treated plants whereas untreated check plants bore heavy infections of the parasites.

Suitability of panagrellus redivivus as a test organism for contact nematicide evaluation. TARJAN, A. C. AND B. E. HOPPER. *Panagrellus redivivus, Diplogaster* sp., *Pungentus pungens, Xiphinema americanum, Meloidogyne incognita, Dolichodorus heterocephalus, Ditylenchus mycophagus,* and *Rotylenchus erythrinae* were compared as to reaction in solutions or emulsions containing 1000 p.p.m. of Triton X-188, Phillips Nematocidal Mixture PN, Triton X-100, Triton X-45, or a dihydroxybenzene. Ten active specimens of each species were immersed in each chemical and observed at ½ hour intervals for 3 hours and again at 24 hours. Each test was replicated 5 times. *Pungentus pungens* and *X. americanum* were inactivated more readily than other species tested. In 10 replicate trials, emulsions containing 250 p.p.m. of either the dihydroxybenzene, an unsaturated aliphatic acid, or chloropicrin were tested against *P. redivivus, M. incognita,* or *D. mycophagus.* No species proved more susceptible to chemical action than others. *P. redivivus* was selected as a preferred test organism because of its large size, ease of culture, short life cycle, and active habit of life. *Panagrellus redivivus* was further tested in emulsions containing 200, 100, and 50 p.p.m. of the dihydroxybenzene and observations were made at 2-hour intervals for 24 hours. Five replicate trials comprised each test. Reduction in chemical concentration resulted in corresponding reductions in number of animals totally inactivated within the designated time limits.

Fungicidal control of Alternaria blight of carrots. WEBER, P. V. V., S. G. YOUNKIN, AND F. L. MERWARTH. Alternaria blight of carrots caused by *Alternaria dauci* frequently causes substantial damage in certain sections of Eastern Pennsylvania and may cause some damage in New Jersey. Fungicide tests have shown statistically significant increases in yield of 7–8 tons per acre with manganese ethylene bisdithiocarbamate, and increases in yield of 5–6 tons per acre with zineb. Defoliation was 38 and 41 per cent, respectively; whereas non-treated plots defoliated 79 per cent. Ziram, Bordeaux, Captan, and a fixed copper gave progressively poorer results in this same test, and defoliation averaged 47, 48, 54, and 55 per cent, respectively, for these materials. All fungicides were used at normally recommended concentrations and applied at 150 gal. per acre. The first applications were made when leafspot first appeared and continued at 10-day intervals until about 2 weeks before harvest. Eight applications were made. Copper-treated plots developed excessively yellow foliage in 2 years' tests and the yield increases and defoliation control obtained were significantly poorer than with Mn. EBD. Polyethylene polysulfide did not increase the efficacy of the fungicides tested.